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I.—On the Law of Error in Target-Shooting. By E. L. De Forest, Watertown, Conn.

The complete expression for the symmetrical law of error in the position of points in a plane is

$$z=\frac{h_1h_2dxdy}{\pi}e^{-(h_1^2x^2+h_2^2y^2)}, \tag{1}$$

where z denotes the probability that an error committed will fall within any small rectangle $dxdy$ whose coördinates, at its middle point, are x and y. The axes should be taken to coincide with the free axes of the group of shot-marks, when these last are regarded as the masses of material points all equal to each other. The origin is at their centre of gravity, and is the point for which the probability z is a maximum. (Compare my article in *The Analyst*, Des Moines, Iowa, vol. viii, p. 73.) Though dx and dy are in strictness infinitesimals, the formula is evidently approximately true when they are regarded as any small finite distances. Points for which z is a given quantity will lie in an ellipse, and all such ellipses are similar and concentric as long as the constants h_1 and h_2 remain the same. These are determined by the relations

$$h_1=\frac{1}{\rho_1\sqrt{2}}, \qquad h_2=\frac{1}{\rho_2\sqrt{2}}, \tag{2}$$

where ρ_1 and ρ_2 are the quadratic mean errors in the x and y directions. If the probability of deviation from the maximum is the same in all directions, then

$$\rho^2=\rho_1^2+\rho_2^2=2\rho_1^2=2\rho_2^2 \tag{3}$$

is the squared q. m. error measured directly from the origin, and

$$h=h_1=h_2=\frac{1}{\rho} \tag{4}$$

is the constant to be introduced in (1). Denoting x^2+y^2 by r^2, (1) is reduced to

$$z=\frac{h^2dx\,dy}{\pi}e^{-h^2r^2}, \tag{5}$$

where the ellipses of equal probability have become circles, and the axes may be taken in any convenient direction. As this formula is the simplest, it is often adopted in discussing the errors of target-shooting. Even when the form (1) is retained, it is robbed of a portion of its generality by assuming that the axes of X and Y are respectively horizontal and vertical, instead of being coincident with the free axes. Although this assumption seems to have been universally made, it has appeared to me to be of doubtful propriety. The only reasons I have seen stated for its adoption are, that errors caused by the wind are horizontal, while those which depend on the range and the force of gravity are vertical. This takes no account of errors produced by other causes, such as defects or peculiarities in the weapon, imperfect sighting, and fatigue or nervousness in the marksman. These may act obliquely, and so far as we know, are as likely to occur in one direction as another.

As a result of accident, it will happen in general, that the centre of gravity of the shot-marks does not exactly coincide with the true point aimed at, namely, the centre of the target. Accidental deviations from the centre of gravity, or from the free axes drawn through it, are thus of the nature of residual errors, while such deviations from the centre of the target, or from axes drawn through it parallel to the former ones, are of the nature of true errors. In any given case, we can compute the amount of probable deviation of the centre of gravity from the centre of the target. If the actual deviation falls within this amount, or does not much exceed it, we may presume that it is purely accidental, and shifting the position of the computed probability surface (1) so as to make its origin coincide with the centre of the target while its coördinate axes remain parallel to their former positions, we shall have the law of probability of error for future shots. But if the actual deviation is far beyond the probable amount, it indicates the probable existence of some constant causes of error, likely to affect future shots in the same way, and the probability surface must not be shifted, unless we also correct the aim of future shots to correspond with it.

It will in general happen also, as the result of accident, that an actual group of shot-marks will be more elongated in one direction than in the direction at right angles to it, so that one of the squared q. m. errors ρ_1^2 ρ_2^2 will be greater than the other, even when the probability of error is really the same in all directions. The constants h_1 and h_2 computed by (2) will thus appear to be different, and the law of error will seem to be as in (1), when it is really of the simpler form (5). But here too, in any given case, we can compute the amount of probable difference between ρ_1^2 and ρ_2^2, supposing it to be accidental. Then if the actual difference falls within this amount, or does not much exceed it, we may presume that the probability of error is really the same in all directions, and that formula (5) may be properly used, the axes being taken horizontal and vertical, simply because those directions are most convenient. On the other hand, if the actual difference between ρ_1^2 and ρ_2^2 is much in excess of its probable value, we must presume that the observed elongation of the group of shot-marks is due to constant causes, likely to have a similar effect on future shots, so that (1) is the most suitable formula to express the law of error, and the axes assumed should be the free axes of the group. It has seemed to me that the question whether formula (1) should be used, and if so, whether the coördinate axes should be made coincident with the free axes, is not a mere matter of opinion, but should be decided by some definite test like the above, applied to an extended set of observations. The most suitable observations for this purpose within my reach are those given by Didion at the close of his *Calcul des Probabilités appliqué au Tir des Projectiles.* Paris, 1858.

His first table gives the positions of 125 shot-marks made by spherical bullets, fired from a rifled pistol at 50 metres, under a charge of one gramme of powder. The weapon was placed on a rest, and aimed at a point 0·430 metres above the centre of the target. The positions of the shot-marks were referred to axes taken horizontally and vertically through that centre. The arith. mean of their ordinates is the ordinate of their centre of gravity, and the arith. mean of their abscissas is its abscissa. We easily find the abscissa u and ordinate v of each shot-mark, referred to axes taken horizontally and vertically through the centre of gravity, and expressed in centimeters. The sums of their squares and of their products are

$$[u^2] = 44211, \qquad [v^2] = 55706, \qquad [uv] = -6655.$$

The angle φ which a free axis makes with the U axis is given by

$$\tan 2\varphi = \frac{2\,[uv]}{[u^2]-[v^2]}. \tag{6}$$

Hence log. tan $2\varphi =$ ·06140, and

$$\varphi = 24^\circ\,31' \qquad \text{or} \qquad \varphi = 114^\circ\,31'. \tag{7}$$

These two values, differing by 90°, represent the inclinations of the two free axes of X and Y to the U axis. Denoting them by φ' and $\varphi'+90^\circ$, the coördinates of a shot-mark referred to the free axes will be

$$x = u\cos\varphi' + v\sin\varphi', \qquad y = v\cos\varphi' - u\sin\varphi', \tag{8}$$

and the sums of their squares are

$$\left.\begin{aligned}[x^2] &= [u^2]\cos^2\varphi' + [v^2]\sin^2\varphi' + [uv]\sin 2\varphi',\\ [y^2] &= [v^2]\cos^2\varphi' + [u^2]\sin^2\varphi' - [uv]\sin 2\varphi',\end{aligned}\right\} \tag{9}$$

from which we readily find

$$[x^2] = 41172 \qquad\qquad [y^2] = 58806.$$

The squared q. m. errors are therefore

$$\rho_1^2 = \frac{[x^2]}{n-1} = 332{\cdot}03, \qquad \rho_2^2 = \frac{[y^2]}{n-1} = 474{\cdot}24, \tag{10}$$

where n is the number of shots.

Since the coördinates of the centre of gravity are the arith. means of those of the shot-marks, the semi-axes of the ellipse of probable error in the position of the centre of gravity are

$$a = 1{\cdot}1774\frac{\rho_1}{\sqrt{n}} = 1{\cdot}92, \qquad b = 1{\cdot}1774\frac{\rho_2}{\sqrt{n}} = 2{\cdot}29. \tag{11}$$

(*Analyst*, viii, p. 77). In the case we are considering, the horizontal and vertical coördinates of the centre of the target referred to the centre of gravity are

$$u = {\cdot}02, \qquad v = 8{\cdot}25,$$

and when referred to the X and Y axes they are by (8)

$$x = 3{\cdot}44, \qquad y = 7{\cdot}50. \tag{12}$$

These values compared with a and b in (11) show that the actual distance of the centre of gravity from the centre of the target is much greater than it probably would be if it were purely accidental. Hence, to represent the probabilities of error in future shots, the surface (1) should in this instance remain with its vertex at the centre of gravity, and not be shifted to the centre of the target, unless the aim is corrected at the same time.

Having thus determined the most probable position of the origin, we wish next to know whether the actual difference between ρ_1^2 and ρ_2^2 is much in excess of what might be expected if it were accidental. Its probable amount may be found approximately as follows. When the q. m. error ε is computed from n observations, the probable error of this determination is known to be

$${\cdot}6745\frac{\varepsilon}{\sqrt{2n}}, \tag{13}$$

and consequently the probable error of ε^2 will be

$${\cdot}6745\varepsilon^2\sqrt{\frac{2}{n}}. \tag{14}$$

Therefore, when ρ_1^2 and ρ_2^2 are computed as in (10), if the probability of error in the x and y directions is really the same, the probable difference between ρ_1^2 and ρ_2^2, occurring from accidental causes, will be

$$\Delta^2 = \cdot 6745 \, \frac{2\varepsilon^2}{\sqrt{n}}, \tag{15}$$

or, if we take approximately $\varepsilon^2 = \frac{1}{2}(\rho_1^2 + \rho_2^2)$,

$$\Delta^2 = \frac{\cdot 6745(\rho_1^2 + \rho_2^2)}{\sqrt{n}}. \tag{16}$$

To apply this to the case in hand, we substitute for ρ_1^2 and ρ_2^2 their numerical values as in (10), and so get for the probable difference

$$\Delta^2 = 48 \cdot 64. \tag{17}$$

The actual value

$$474 \cdot 24 - 332 \cdot 03 = 142 \cdot 21,$$

is so much larger that we are obliged to conclude that in this case it is probably not an accidental but a real difference, likely to affect the distribution of future shots in the same way. Hence (1) is the proper formula to use, and the coördinate axes ought to be taken not horizontally and vertically, but coincident with the free axes of the group of shot-marks.

Didion also gives a table of the positions of the shot-marks made by firing a pistol, apparently similar and with equal charge, 250 times at 100 metres distance, aiming at a point 1·47 m. above the centre of the target. By the same procedure as before, we find

$$[u^2] = 1392560, \qquad [v^2] = 1742890, \qquad [uv] = -301930,$$

where u and v are expressed in centimetres. The inclinations of the free axes to the U axis then are by (6)

$$\varphi' = 29^\circ\, 56', \qquad \varphi' + 90^\circ = 119^\circ\, 56', \tag{18}$$

and with coördinates referring to these axes we have by (9)

$$[x^2] = 1218600, \qquad [y^2] = 1916800,$$

so that the squared q. m. errors are by (10)

$$\rho_1^2 = 4894 \cdot 0, \qquad \rho_2^2 = 7698 \cdot 0. \tag{19}$$

The semi-axes of the ellipse of probable error in the position of the centre of gravity are by (11)

$$a = 5 \cdot 21, \qquad b = 6 \cdot 53. \tag{20}$$

But the horizontal and vertical coördinates of the centre of the target are

$$u = 1 \cdot 72, \qquad v = 15 \cdot 82,$$

which referred to the free axes become

$$x = 9{\cdot}38, \qquad y = 12{\cdot}85. \qquad (21)$$

Comparing these with (20), we see that the actual distance of the centre of gravity from the centre of the target is too great to be considered accidental, and we infer as in the former case, that to represent the probabilities of future shots, the vertex of the probability surface should remain at the centre of gravity, and not be changed to the centre of the target, unless the aim is also changed.

The probable difference in this case between ρ_1^2 and ρ_2^2 is by (16)

$$\Delta^2 = 537{\cdot}2. \qquad (22)$$

The actual difference however is

$$7698{\cdot}0 - 4894{\cdot}0 = 2804{\cdot}0,$$

a value so much greater that we are obliged to conclude that it is probably not accidental.

This is what might be expected from the results already obtained with the same kind of weapon at shorter range. If there is really a greater liability to error in one direction than in another, it will naturally show itself at all ranges, with only such differences as might occur by accident. The angles which the direction of greatest error makes with the X axis at the two ranges here considered

$$\varphi' + 90° = 114° \, 31' \quad \text{and} \quad \varphi' + 90° = 119° \, 56',$$

are not very different from each other.

Didion finally gives the coördinates of the trajectories of 100 cannon balls fired, under constant charges and angles of elevation, at distances of 200, 400 and 600 metres. The heights are reckoned, not from the centre of a target, but from the plane of the platform on which the gun-carriage stands. By an easy reduction, we get the coördinates u and v referred to axes taken horizontally and vertically through the centre of gravity, and expressed in metres. For 200 metres range, we find

$$[u^2] = 11{\cdot}82, \qquad [v^2] = 16{\cdot}39, \qquad [uv] = -0{\cdot}18,$$

and (6) gives

$$\varphi' = 2° \, 15', \qquad \varphi' + 90° = 92° \, 15'. \qquad (23)$$

Then by (9),

$$[x^2] = 11{\cdot}81, \qquad [y^2] = 16{\cdot}40,$$

and by (10),

$$\rho_1^2 = {\cdot}1193, \qquad \rho_2^2 = {\cdot}1657.$$

The probable difference between ρ_1^2 and ρ_2^2 is by (16)

$$\Delta^2 = {\cdot}0192. \qquad (24)$$

The actual difference is greater, being

$$\cdot 1657 - \cdot 1193 = \cdot 0464.$$

We omit the discussion for the 400 and 600 metre ranges, since the shots here are apparently the same as those used at 200 metres, and the differences in relative position for the same shot at different ranges seem to be largely due to accidental variations in the projejectile or the powder. From the three series of trials retained, namely, two of pistol shots and one of cannon shots, it appears that the test requires us to use formula (1), and to make the coördinate axes coincide with the free axes. This therefore, it seems to me, had better be generally done when accuracy is desired, unless indeed our results should hereafter be invalidated by those of other and more extended experiments.

We might also inquire what are the probable values, arising from accidental causes, of the cubes of the c. m. inequalities in x and y, when the law of error in either direction is suspected to be unsymmetrical. This question finds an answer in my article on the Unsymmetrical Probability Curve, *Analyst*, vol. x, p. 74. See also *Trans. of the Conn. Academy*, vol. vi, part 1. For determining a_1 and a_2 the easiest way will perhaps be to compute the values of $[u^3]$, $[v^3]$, $[u^2v]$ and $[uv^2]$. Then from (8) we have

$$\left.\begin{aligned}
[x^3] &= [u^3]\cos^3\varphi' + [v^3]\sin^3\varphi' \\
&\qquad + 3\sin\varphi'\cos\varphi'([u^2v]\cos\varphi' + [uv^2]\sin\varphi') \\
[y^3] &= [v^3]\cos^3\varphi' - [u^3]\sin^3\varphi' \\
&\qquad - 3\sin\varphi'\cos\varphi'([uv^2]\cos\varphi' - [u^2v]\sin\varphi')
\end{aligned}\right\} \quad (25)$$

and a_1 and a_2 are obtained like a in *Analyst*, ix, p. 161. For example, from the first table here tried we get

$$[u^3] = 322100, \qquad [u^2v] = -9600,$$
$$[v^3] = 264700, \qquad [uv^2] = 37400,$$

and consequently by (7) and (25)

$$[x^3] = 269207, \qquad [y^3] = 133301,$$

and the cubed inequalities are

$$\zeta_1^3 = \frac{n[x^3]}{n^2-1} = 2153\cdot 8, \qquad \zeta_2^3 = \frac{n[y^3]}{n^2-1} = 1066\cdot 5. \qquad (26)$$

From accidental causes alone, they would probably be something like

$$\left.\begin{aligned}
(\zeta_1^3) &= \pm \cdot 6745\rho_1^3\sqrt{\frac{15}{n}} = \pm 1413\cdot 7, \\
(\zeta_2^3) &= \pm \cdot 6745\rho_2^3\sqrt{\frac{15}{n}} = \pm 2413\cdot 1.
\end{aligned}\right\} \quad (27)$$

The actual value of ζ_2^3 falls far within the probable amount, and that of ζ_1^3 does not so much exceed the probable one as to make us confident that it is anything more than accidental. But if both the inequalities were taken into account, we should have

$$\left.\begin{array}{ll} a_1 = 2\rho_1^2 \div \zeta_1^3 = \cdot30832 & b_1 = \rho_1^2 = 332\cdot03 \\ a_2 = 2\rho_2^2 \div \zeta_2^3 = \cdot88934 & b_2 = \rho_2^2 = 171\cdot21 \end{array}\right\} \quad (28)$$

and the equation of the surface will be of the same form as in formula (34) of my article in the *Transactions.* The sub-index of the first b_1 should fall inside the bracket, instead of outside as there printed.

II.—Extensions of certain Theorems of Clifford and of Cayley in the Geometry of n Dimensions. By Eliakim Hastings Moore, jr., Denver, Colorado.

I. General Theorems.

Clifford, at the beginning of his "Classification of Loci" (*Mathematical Papers*, p. 305–331), proves the following theorems:

A. Every proper curve of the n^{th} order is in a flat space of n dimensions or less.

B. A curve of order n in flat space of k dimensions (and no less) may be represented, point for point, on a curve of order $n-k+2$ in a plane, whence

C. A curve of order n in flat space of n dimensions (and no less) is always *unicursal.*

These theorems may be extended. Clifford's nomenclature[*] and methods of proof are adhered to throughout.

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[*] For the convenience of the reader who may not have at hand a copy of Clifford's *Mathematical Papers*, the definitions (p. 305–6) are given here.

"By a *curve* we mean a continuous one-dimensional aggregate of any sort of elements, and therefore not merely a curve in the ordinary geometrical sense, but also a singly infinite system of curves, surfaces, complexes, &c., such that one condition is sufficient to determine a finite number of them. The elements may be regarded as determined by k coordinates; and then, if these be connected by $k-1$ equations of any order, the curve is either the whole aggregate of common solutions of these equations, or, when this breaks up into algebraically distinct parts, the curve is one of these parts. It is thus convenient to employ still further the language of geometry, and to speak of such a curve as the complete or partial intersection of $k-1$ loci in flat space of k dimensions, or, as we shall sometimes say, in a k-flat. If a certain number, say h, of the equations are linear, it is evidently possible by a linear transformation to make these equations equate h of the coordinates to zero; it is then convenient to leave these coordinates out of consideration altogether, and only to regard the remaining $k-h-1$ equations between $k-h$ coordinates. In this case the curve will, therefore, be regarded as a curve in a flat space of $k-h$ dimensions. And, in general, when we speak of a curve as in flat space of k dimensions, we mean that it cannot exist in flat space of $k-1$ dimensions.

* * * By a *surface* we shall mean, in general, a continuous two-dimensional aggregate (which may also be called a *two-spread* or *two-way locus*) of any elements whatever, curves, surfaces, complexes, &c., defined by the whole or a portion of the system of solutions of $k-2$ equations among k coordinates. We shall assume that none of these equations are linear, and then shall speak of the surface as in a flat

Theorem A. *Every proper* r-*spread of the* n" *order is in a flat space of* n + r — 1 *dimensions or less.*

For through $n+1$ points of the r-spread we can draw an n flat, R_n; this meets the r-spread S_r in a number of points greater than its order, and, therefore, contains a curve, or 1-spread S_1 of the r spread S_r.

An $(n+1)$flat R_{n+1} drawn through this n-flat R_n and an external point of the r-spread S_r, for a similar reason, contains a 2-spread S_2 of the r-spread S_r.

Thus, finally, there is reached an $(n+r-1)$flat R_{n+r-1}, which completely contains the r-spread S_r.

An r-spread of order n, say $S_{r,n,k}$, may lie in a flat space of k dimensions, where $k \leqq n+r-1$; when $k=n+r-1$, the $S_{r,n,n+r-1}$ may be called a *full skew* r-spread of order n.

Theorem B. *An* r-*spread of order* n *in a flat space of* k *dimensions (and no less), say* $S_{r,n,k}$, *may be represented, point for point, on an* r-*spread of order* n—k+r+1 *in an* (r+1)*flat*, $S_{r,n-k+r+1,r+1}$.

Join P, an arbitrary fixed point, to Q, a variable point, both being on the r-spread; the resulting $(r+1)$spread S_{r+1} is of the order $n-1$; for a $(k-r)$flat R_{k-r} through P meets the r-spread S_r elsewhere in $n-1$ points Q, and, therefore, meets the S_{r+1} in the $n-1$ lines PQ, i. e., in a curve of order $n-1$. Each line PQ meets a fixed $(k-1)$flat R_{k-1} in a point Q′ corresponding to Q; the $(r+1)$spread S_{r+1} meets the fixed flat R_{k-1} in an r spread of order $n-1$. The r-spread of the n^{th} order in k-flat $S_{r,n,k}$ is thus projected into one of order $n-1$ in a $(k-1)$flat, $S_{r,n-1,k-1}$. A second projection from an arbitrary point upon a fixed $(k-2)$flat R_{k-2} gives an r-spread of order $n-2$ in a $(k-2)$flat, $S_{r,n-2,k-2}$.

Thus, finally, after $k-r+1$ successive projections the original $S_{r,n,k}$ is represented, point for point, on an r-spread of order $n-k+r+1$ in an $(r+1)$flat, $S_{r,n-k+r+1,r+1}$.

But the result may be reached at once. Through any $k-\overline{r}+1$ fixed points P of the r-spread and a variable point Q pass a $(k-r+1)$flat $R_{k-\overline{r+1}}$, cutting a fixed $(r+1)$flat R_{r+1}, in a point Q′ corresponding to Q. Thus the $S_{r,n,k}$ is represented, point for point,

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space of k dimensions. We shall in certain cases go further, and speak of an h-spread or h-way locus, viz: a locus determined by the whole or an algebraically separate portion of the system of solutions of $k-h$ equations among k coordinates; if none of these equations are linear, the h-way locus will be said to be in k dimensions."

A *proper* curve or spread is one which does not break up into two or more algebraically distinct parts on an r-spread in the $(r+1)$flat, $S_{r,(n-k+r+1),r+1}$, the *order* of which is $n-k+r+1$, since a $(k-r)$flat R_{k-r}, through the $k-r+1$ fixed points P meets the r-spread $S_{r,n,k}$ in $n-k+r+1$ additional points Q which correspond to the $n-k+r+1$ points Q′ in which the line of intersection of the R_{k-r} with the fixed R_{r+1} meets the projected r-spread $S_{r,n-k+r+1,r+1}$.

THEOREM C. A quadric r-spread is in an $(r+1)$flat, and is *unicursal*, its points having (e. g., by projection from a point on it) a one-one correspondence with those of an r-flat R_r. Hence, a *full skew* r-spread of order n, $S_{r,n,n+r-1}$, is *always unicursal*, since, by theorem B, it may be represented on an r-spread of order $n-k+r+1=2$ in an $(r+1)$flat. Not only so, but *every flat* section of a full skew r-spread is *itself* a full skew spread, and, therefore, *unicursal*. For an s-flat R_s cuts a full skew $S_{r,n,k\ (k+1=r+n)}$ in an

$$\begin{aligned} S_{r+s-k,n,s} &\equiv S_{r',n',k'}, \text{ which is } \textit{full skew}, \\ \text{since}\quad & k'+1=r'+n', \\ \text{i. e.,}\quad & s+1=(r+s-k)+n, \text{ using the given} \\ \text{relation,}\quad & k+1=r+n. \end{aligned}$$

II. FULL SKEW TWO-SPREADS.

1. *The abbildung-system.*

The full skew two-spread of order m in R_{m+1}, $S_{2,m,m+1}$, is unicursal; the curve of intersection with any m-flat R_m, $S_{1,m,m}$ is also unicursal (I, C); an R_{m-1}, the intersection of two R_m, and so the axis of a pencil of R_m, meets the two-spread in m points (m being the order of the spread); further, in the R_{m+1}, the all-including flat, there are ∞^{m+1} m-flats R_m, i. e., $m+2$ asyzygetic R_m. Hence, there is a representation or *Abbildung* of the $S_{2,m,m+1}$, point for point, on a plane $y_1y_2y_3$, $\mathfrak{R}_2$; to an R_m-section corresponds a *unicursal* curve, say of order n, $\mathfrak{C}^n$; to the m points of intersection of an R_{m-1} correspond m points of intersection of a pencil of curves $\mathfrak{C}^n$; the Abbildung of the $(m+1)$ply infinite system of R_m-intersections $S_{1,m,m}$ is the system of curves $\mathfrak{C}^n$, $(m+1)$ply infinite or linearly derivable from $m+2$ asyzygetic curves of the system, all of which are unicursal and have the equivalent of n^2-m common points of intersection, say *base-points* of the system. A Cremona transformation may be found which will change an abbildung-system $\mathfrak{C}^n$ of the spread $S_{2,m,m+1}$ into any other abbildung-system $\mathfrak{C}^{n'}$ of the same spread, since there is a one-one correspondence between the points of the two coincident planes $\mathfrak{R}_2$ containing the two abbildung-systems. There is, therefore, no loss of generality in assuming as the abbildung-system, the system of curves $\mathfrak{C}^m$ having as base-points an $(m-1)$ple pt. $\mathfrak{O}$ and $m-1$ other points, $\mathfrak{P}_1, \ldots \mathfrak{P}_{m-1}$: the conditions given above are satisfied by this system; for the curves are unicursal; the asyzygetic number is $2m+1-m-1=m+2$; the base-points are equivalent to

$$(m-1)^2+(m-1)=m(m-1)=m^2-m=n-m$$

common points of intersection.

This abbildung-system may be simplified. Transform the plane $\mathfrak{R}_2$ by a quadric transformation, having $\mathfrak{O}$, $\mathfrak{P}_{m-1}$ and $\mathfrak{P}_m$, as fundamental points.

A curve $\mathfrak{C}^m$, of order m, passing through the two fundamental points $\mathfrak{P}_{m-1}$, $\mathfrak{P}_m$, and having an $(m-1)$ple pt at $\mathfrak{O}$, the third fundamental point, and passing through $m-3$ fixed points $\mathfrak{P}_1 \ldots \mathfrak{P}_{m-3}$, is transformed into a curve $\mathfrak{C}'$ of order $m-1=2.m-(m-1+1+1)$, having an $(m-2)$ple pt. at $\mathfrak{O}$, (since the line $\mathfrak{P}_{m-1}\mathfrak{P}_m$ meets the $\mathfrak{C}^m$ in $m-2$ additional pts.), passing through the $m-3$ fixed points $\mathfrak{P}'_1 \ldots \mathfrak{P}'_{m-3}$ corresponding to the pts. $\mathfrak{P}_1 \ldots \mathfrak{P}_{m-3}$, and not passing through $\mathfrak{P}_{m-1}$ [$\mathfrak{P}_{m-2}$], (since the line $\mathfrak{O}\mathfrak{P}_{m-2}$ [$\mathfrak{O}\mathfrak{P}_{m-1}$] does not meet the $\mathfrak{C}^m$ except in $\mathfrak{O}$ and $\mathfrak{P}_{m-2}$ [$\mathfrak{O}$ and $\mathfrak{P}_{m-1}$]).

Thus such a quadric transformation reduces by unity the order of the curves of the abbildung-system, and deletes two of the base-points. By r such quadric transformations, the abbildung-system of curves $\mathfrak{C}^m$, with $\mathfrak{O}$, an $(m-1)$ple point, and $m-1$ other points $\mathfrak{P}$, as base-points, is changed into a system of curves $\mathfrak{C}^{m-r}$ with $\mathfrak{O}$, an $(m-r-1)$ple point, and $m-1-2r$ other points $\mathfrak{P}$, as base-points. The simplest abbildung-systems are, evidently,

(*a*) m even$=2m'$, $r=m'-1$ $\mathfrak{C}^{m'+1}$ with $\mathfrak{O}$ as m'ple point
and through *one* pt. $\mathfrak{A}$.

(*b*) m odd$=2m''+1$, $r=m''$. $\mathfrak{C}^{m''+1}$ with $\mathfrak{O}$ as m''ple point.

2. *The canonical form of the equations of the* $S_{2,m,m+1}$.

Let the X_s $(s=1, \ldots m+3)$ be the homogeneous coordinates in R_{m+1} and the y_s $(s=1, 2, 3)$ be the homogeneous coordinates in $\mathfrak{R}_2$.

(*a*) m even$=2m'$. $\mathfrak{O}$ is $y_1, y_2, y_3=0, 0, 1$; $\mathfrak{A}$ is $y_1, y_2, y_3=1, 0, 0$.

By the simplified abbildung-system set

$$\begin{aligned}
&X_1:X_2:X_3:\ldots\ldots\ldots\ldots\ldots:X_{m'-1}:X_{m'}:X_{m'+1}:\\
&X_{m'+2}:X_{m'+3}:X_{m'+4}:\ldots\ldots\ldots\ldots\ldots:\underset{m+1}{X_{2m'+1}}:\underset{m+2}{X_{2m'+2}}::\\
&y_1^{m'}y_2:y_1^{m'-1}y_2^2:y_1^{m'-2}y_2^3:\ldots\ldots\ldots:y_1^2y_2^{m'-1}:y_1y_2^{m'}:y_2^{m'+1}:\\
&y_3[y_1^{m'}:y_1^{m'-1}y_2:y_1^{m'-2}y_2^2:\ldots\ldots\ldots\ldots:y_1y_2^{m'-1}:y_2^{m'}].
\end{aligned}$$

Setting $y_2=ty_1$ and $y_3=uy_2$, this becomes

$$\begin{matrix} X_1:X_2:X_3:\,.\,.:X_{m'-1}:X_{m'}:X_{m'+1}:X_{m'+2}:X_{m'+3}:\,.\,.:X_{m+1}:X_{m+2}:: \\ 1:t:t^2:\,.\,.\,.\,.\,.:t^{m'-2}:t^{m'-1}:t^{m'}: \qquad u\,(1:t:\,.\,.\,.\,.\,.:t^{m'-1}:t^{m'}); \end{matrix}$$

whence the plexus of equations

$$\left|\begin{matrix} X_1, X_2, X_3, \,.\,.\,.\,.\,.\, X_{m'-1}, X_{m'}, & X_{m'+2}, X_{m'+3}, X_{m'+1}, \,.\,.\,.\,.\,.\, X_{m+1} \\ X_2, X_3, \,.\,.\,.\,.\,.\,.\,.\, X_{m'}, X_{m'+1}, & X_{m'+3}, X_{m'+1}, \,.\,.\,.\,.\,.\, X_{m+1}, X_{m+2} \end{matrix}\right\|=0$$

(*b*) m odd $=2m''+1$. $\qquad\mathfrak{O}$ is $y_1, y_2, y_3=0, 0, 1$.

In accordance with the simplified abbildung-system, set

$$\begin{matrix} X_1:X_2:X_3:\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.:X_{m''+1}:X_{m''+2}: \\ X_{m''+3}:X_{m''+4}:X_{m''+5}:\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.:X_{\substack{2m''+2\\ m+1}}:X_{\substack{2m''+3\\ m+2}}:: \\ y_1^{m''+1}y_2^{0}:y_1^{m''}y_2^{1}:y_1^{m''-1}y_2^{2}:\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.\,.:y_1^{1}y_2^{m''}:y_1^{0}y_2^{m''+1}: \\ y_3\,[y_1^{m''}y_2^{0}:y_1^{m''-1}y_2^{1}:y_1^{m''-2}y_2^{2}:\,.\,.\,.\,.\,.\,.\,.:y_1^{1}y_2^{m''-1}:y_1^{0}y_2^{m''}], \end{matrix}$$

or, setting $\qquad y_2=ty_1$ and $y_3=uy_1$,

$$\begin{matrix} X_1:X_2:X_3:\,.\,.\,.\,.\,.:X_{m''+1}:X_{m''+2}:\,.\,.\,.\,.\,.:X_{m+1}:X_{m+2}:: \\ 1:t:t^2:t^3:\,.\,.\,.\,.:t^{m''}:t^{m''+1}:u\,[1:t:t^2:\,.\,.\,.\,.:t^{m''-1}:t^{m''}]; \end{matrix}$$

whence the plexus of equations

$$\left\|\begin{matrix} X_1, X_2, X_3, \,.\,.\,.\,.\,.\, X_{m''+1}, & X_{m''+3}, X_{m''+4}, X_{m''+5}, \,.\,.\,.\,.\,.\, X_{m+1} \\ X_2, X_3, \,.\,.\,.\,.\, X_{m''+1}, X_{m''+2}, & X_{m''+4}, X_{m''+5}, \,.\,.\,.\,.\,.\,.\, X_{m+1}, X_{m+2} \end{matrix}\right\|=0$$

In each case the canonical form exhibits the $S_{2,m,m+1}$ as the locus of the line of intersection of corresponding m-flats R_m of m projective pencils of R_m; $\qquad X_2-tX_1=0, \quad X_3-tX_2=0$, &c.

In fact, these right lines on and ruling the $S_{2,m,m+1}$ correspond to the right lines on the plane $\mathfrak{R}_2$ through the multiple point $\mathfrak{O}$; $y_2-ty_1=0$ is met by a $C^{m'+1}$ or $C^{m''+1}$ (which corresponds to an R_m-section of $S_{2,m,m+1}$) in only *one* point.

A full skew *curve* O of order $\begin{Bmatrix}(a)\ m' \\ (b)\ m''\end{Bmatrix}$ corresponds to the point $\mathfrak{O}$. $\mathfrak{O}$ is $(0, 0, 1)$; or $u=\infty$, t indeterminate; and, therefore, $\mathfrak{O}$ corresponds

$$(a)\ \text{to}\ X_1=X_2=\,.\,.\,.\,.=X_{m'+1}=0 \qquad \left\|\begin{matrix} X_{m'+2}, X_{m'+3}, \,.\,.\,.\,.\,.\,.\, X_{m+1} \\ X_{m'+3}, \,.\,.\,.\,.\,.\,.\, X_{m+1}, X_{m+2} \end{matrix}\right\|=0$$

$$(b)\ \text{to}\ X_1=X_2=\,.\,.\,.\,.=X_{m''+2}=0 \qquad \left\|\begin{matrix} X_{m''+3}, X_{m''+5}, \,.\,.\,.\,.\,.\, X_{m+1} \\ X_{m''+4}, \,.\,.\,.\,.\,.\,.\, X_{m+1}, X_{m+2} \end{matrix}\right\|=0$$

or the (unicursal) curve $\begin{Bmatrix}(a)\ m' \\ (b)\ m''\end{Bmatrix}$ in an $\begin{matrix}m'\text{-} \\ m''\text{-}\end{matrix}$flat. Cf. Clifford, p. 310.

(*a*) A *line* A corresponds to the *point* $\mathfrak{A}$. For $\mathfrak{A}$ is (1, 0, 0); or u indeterminate, $t=0$; and, therefore, $\mathfrak{A}$ corresponds to

$$X_2=X_3=\,.\,.\,.\,.\,.=X_{m'+1}=0 \qquad X_{m'+3}=X_{m'+4}=\,.\,.\,.\,.\,.=X_{m+2}=0,$$

m m-flats R_m in R_{m+1} intersect in R_1, a right line.

A *point* corresponds to the *line* $\mathfrak{O}\mathfrak{A}$, viz: the intersection of the full-skew curve O and the line A. $y_1=0$; or $u=\infty$, $t=0$;

$$X_1=X_2=\ldots\ldots=X_{m+1}=0, \qquad X_{m+2}=X_{m+3}\;\ldots\ldots\;X_{m+n}\;\;0.$$

3. *Tangent and osculating flats.*

On the two-spread let Q be a point and C a curve passing through Q; there are at Q a tangent line R_1, and osculating 2 flat R_2, osculating R_3 . . . R_t to the curve C, determined by 2, 3 . . . $(t+1)$ consecutive points (Q included) of the curve; the osculating t-flat R_t meets the curve C in $t+1$ points at P. The tangent plane R_2 at Q to the two-spread is the locus of tangent lines R_1 to all curves C through Q; every m-flat R_m through this tangent plane R_2 meets the spread in a curve having a double-pt. at Q.

Suppose there were an s-flat R_s such that every R_m through it met the two-spread in a curve having a $(t+1)$ple pt. at Q. Take *any* curve C through the point P; *every* R_m through the R_s meets the curve C in $t+1$ points at Q, and, therefore, contains the osculating R_t to C at Q; hence the R_s contains the osculating t-flats R to *all* curves C through Q, or say it meets the two-spread at t consecutive points in every direction from Q.

In the case of this *full skew* two-spread it will be shown that at every pt. Q there is an osculating R_{2t}, containing the osculating R_t to every curve through Q; but probably $s=2t$ for the *general* two-spread in any number of dimensions, R_k; $(2t\leqq k$, of course).

Considerations from the abbildung-system.

The general abbildung-system (cf. §1) of curves $\mathfrak{C}^m$ has as base-pts. the $(m-1)$ple pt. $\mathfrak{O}$, and the $m-1$ pts. $\mathfrak{P}$; there are $m+2$ asyzygetic curves of the system. Consider those curves of the system which have a $(t+1)$ple pt. at $\mathfrak{Q}$, say $\mathfrak{C}^m(\mathfrak{Q}^{t+1})$; these correspond to the R_m-sections of the two spread which have a $(t+1)$ple pt. at Q. Such a curve $\mathfrak{C}^m(\mathfrak{Q}^{t+1})$ includes the line $\mathfrak{O}\mathfrak{Q}$ t times, and, besides, a supplementary curve $\mathfrak{C}^{m-t}(\mathfrak{Q})$, passing through $\mathfrak{Q}$ and the $m-1$ pts. $\mathfrak{P}$ and having an $(m-t-1)$ple pt. at $\mathfrak{O}$. For say the curve $\mathfrak{C}^m(\mathfrak{Q}^{t+1})$ includes the line $\mathfrak{O}\mathfrak{Q}$ x times, and, therefore, also a supplementary curve $\mathfrak{C}^{m-x}(\mathfrak{Q}^{t+1-x})$ with $(t+1-x)$ple pt. at $\mathfrak{Q}$ and $(m-x-1)$ple point at $\mathfrak{O}$; the line $\mathfrak{O}\mathfrak{Q}$ meets the supplementary curves in $(m-x-1)+(t+1-x)=m+t-2x$ pts. and will be again thrown off, if $m-x<m+t-2x$, i. e., if $x<t$. The asyzygetic number for the system of supplementary curves $\mathfrak{C}^{m-t}(\mathfrak{Q})$ is $(\overline{2m-2t+1}-\overline{m}-\overline{1}-1=)$ $m-2t+1$. Hence there are $m-2t+1$ asyzygetic m-flats R_m meeting

the two-spread in curves with Q as $(t+1)$ple pt.; these R_m in the all-including flat R_{m+1} meet in an $(m+1)-(m-2t+1)=2t$-flat R_{2t} which is the osculating $2t$-flat at Q, which includes the osculating t-flat R_t at Q to every curve through Q; i. e., which meets the two-spread at t consecutive points in every direction from Q; i. e., which contains the osculating $2(t-1)$flats K_{2t-2} at all points immediately adjacent to Q.

What is the locus of osculating $2t$-flats at points Q along a generator τ of the two-spread?

If a curve $\mathfrak{C}^m$ degenerates into $\mathfrak{D}^{(t)}$, say the line τ, taken $t+1$ times and a supplementary curve $\mathfrak{C}^{m-t-1}$ ($\mathfrak{O}$ as $\overline{m-t-2}$ple pt. and through the $m-1$ pts. $\mathfrak{P}$) the R_m cutting the two-spread in the corresponding section will contain the osculating $2t$-flat at every point Q along the generator τ. The asyzygetic number for the system of supplementary curves $\mathfrak{C}^{m-t-1}$ is $(2m-2t-1)-(m-1)=m-2t$. Hence there are $m-2t$ asyzygetic m-flats R_m' meeting the two-spread in the generator τ taken $(t+1)$ times; these in the R_{m+1} meet in an $(m+1)-(m-2t)=(2t+1)$flat R_{2t+1}, say the osculating R_{2t+1} along τ^{t+1} the $(t+1)$ple generator τ, which is the locus of the osculating R_{2t} at the points Q along the generator τ.

The equations of the osculating flats; the orders of the spreads they generate.

(a) m even $=2m'$. Cf. §2, the simplified abbildung-system; the equations in t.

The equations correspond to $m-2t$ asyzygetic curves $\mathfrak{C}^{m'+1}$ including the line τ $t+1$ times; and to the one other asyzygetic curve of the system including the line τ t times and passing through the point $Q \equiv \tau v$.

$t=0$. R_1, the line τ.

$$\begin{array}{ll}
\tau X_1 - X_2 \quad = 0, & \tau X_{m'+2} - X_{m'+3} = 0, \\
\tau X_2 - X_3 \quad = 0, & \tau X_{m'+3} - X_{m'+4} = 0, \\
\quad\vdots & \quad\vdots \\
\tau X_{m'} - X_{m'+1} = 0, & \tau X_{m+1} - X_{m+2} = 0.
\end{array}$$

$2m'(=m)$ R_m meeting in R_1, the line τ.

The additional R_m, $vX_1 - X_{m'+2}=0$, determines the point τv on the line τ.

$t=1$. R_3, the osculating R_{2t+1}, along τ^{t+1}, the locus of tangent planes of points v along the line τ.

$$\begin{aligned}
&\tau^2X_1-2\tau X_2+X_3=0, && \tau^2X_{m'+2}-2\tau X_{m'+3}+X_{m'+4}=0,\\
&\tau^2X_2-2\tau X_3+X_4=0, && \tau^2X_{m'+3}-2\tau X_{m'+4}+X_{m'+5}=0,\\
&\tau^2X_{m'-1}-2\tau X_{m'}+X_{m'+1}=0, && \tau^2X_m-2\tau X_{m+1}+X_{m+2}=0.
\end{aligned}$$

$2(m'-1)=m-2$ R_m meeting in R_3.

The additional R_m, $v(\tau X_1-X_2)-(\tau X_{m'+2}-X_{m'+3})=0$, determines the tangent plane R_2 at the point τv.

And so, in general, the law of formation is clear.

The osculating R_{2t+1} along τ^{t+1}, the locus of the osculating R_{2t} of points v along the line τ. The equations may be written

$$\begin{aligned}
&X^1(\tau-X^1)^{t+1}=0, && X^{m'+2}(\tau-X^1)^{t+1}=0,\\
&X^2(\tau-X^1)^{t+1}=0, && X^{m'+3}(\tau-X^1)^{t+1}=0,\\
&\quad\vdots && \quad\vdots\\
&X^{m'-t}(\tau-X^1)^{t+1}=0, && X^{m-t+1}(\tau-X^1)^{t+1}=0;
\end{aligned}$$

where, after the binomial expansion and multiplication by the external factor, the exponents of the X are to be exchanged for corresponding subscripts; X^r is to be changed to X_r.

$2(m'-t)=m-2t$ R_m meeting in R_{2t+1}.

The additional R_m, $(vX^1-X^{m'+2})(\tau-X^1)^t=0$ (the exponents becoming subscripts, as above), determines the osculating $2t$-flat R_{2t} at the point τv.

The osculating R_{2t-1} along τ^t lies in the osculating R_{2t+1} along τ^{t+1}; the two R_m, $X^1(\tau-X^1)^t=0$, $X^{m'+2}(\tau-X^1)^t=0$, with the $m-2t$ equas. of the R_{2t+1} written above are easily seen to be equivalent to the $m-2(t-1)$ equations of the R_{2t-1},

$$\begin{aligned}
&X^1(\tau-X^1)^t=0 && X^{m'+2}(\tau-X^1)^t=0\\
&\quad\vdots && \quad\vdots\\
&X^{m'-t+1}(\tau-X^1)^t=0 && X^{m-t+2}(\tau-X^1)^t=0.
\end{aligned}$$

Thus the equations show that the singly infinite system of osculating $2t$-flats R_{2t} at points v along the generator τ lie in the osculating $(2t+1)$flat R_{2t+1} along τ^{t+1}; and, at the same time, form a *pencil* of R_{2t} in the R_{2t+1} having as an *axis* the osculating $(2t-1)$ flat R_{2t-1} along τ^t; and the osculating flats of the pencil are *homographic** with their points of osculation along the generator τ.

Compare the well known theorem: Salmon; *Geometry of Three*

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* The only equation introducing v is *linear* in v.

Dimensions, §459; "the anharmonic ratio of four tangent planes passing through a generator of a ruled surface is equal to that of their four points of contact."

(*b*) m odd $= 2m'' + 1$.

There is one more equation in each case from the first group of coordinates, $X_1 \quad {}_{m+2}$, than from the second group, $X_{m''+1}$ . . X_{m+2}; the v equation is of exactly the same nature; the conclusions stated above hold equally for m odd or even.

As the line τ generates the two-spread $S_{2,m,m+1}$ the osculating R_{2t+1} along τ^{t+1} generates a $(2t+2)$spread of order $(t+1)$ $(m-2t)$ $S_{2t+2,(t+1)(m-2t),m+1}$.

Let the $m-2t$ asyzygetic R_m determining R_{2t+1} in terms of τ be A_m, A_{m-1}, ... A_{2t+1}; the A involve τ to the power $t+1$; let $A_{m,s}$, $A_{m-1,s}$, . . . $A_{2t+1,s}$, be what the A become when the coordinates of a point P_s in the $(m+1)$flat are substituted for the current coordinates. The $(2t+2)$spread is met by an $(m-2t-1)$flat in say x points P_s; any point in the $(m-2t-1)$flat may be given in terms of $m-2t$ asyzygetic points P_m . . . . P_{2t+1};

$$\text{say } P_s = \lambda_m P_m + \lambda_{m-1} P_{m-1} + \ . \ . \ . \ + \lambda_{2t+1} P_{2t+1},$$
$$\text{i. e., } X_{1,s} = \lambda_m X_{1,m} + \lambda_{m-1} X_{1,m-1} + \ . \ . \ . \ + \lambda_{2t+1} X_{1,2t+1}, \text{ etc.}$$

Substituting the coordinates of P_s in the $m-2t$ A_r, and eliminating from the $m-2t$ $A_{r,s}$ the $m-2t$ λ which enter homogeneously in the first degree, we have the determinant of the order $m-2t$

$$\begin{array}{lll} A_{m,n} & {}_{m,m-1}, & \\ A_{m-1,m}, & A_{m-1,m-1}, & . \ . \ A_{m+1,2t+1} \\ & & \\ & & \\ A_{2t+1,m}, & A_{2t+1,m-1}, & . \ . \ A_{2t+1,2t+1} \end{array} = 0,$$

an equation of degree $(t+1)(m-2t)$ in τ; for each value of τ, there is one set of values of the λ, one point P_s. Hence the order of the $(2t+2)$spread, the locus of osculating R_{2t+1}, is $(t+1)(m-2t)$, as stated. Through a point P_s an osculating R_{2t+1} along τ^{t+1} may be drawn; this contains the osculating R_{2t-1} along τ^t; the R_{2t} joining the point P_s with this R_{2t-1} is the osculating R_{2t} at some point v of the line τ. This may be expressed thus; through an R_{m-2t-1} may be passed $(t+1)(m-2t)$ m-flats R_m which meet the spread in a $(t+1)$ple line τ; and $(t+1)(m-2t)$ $(m-1)$flats R_{m-1} which meet the spread in a $(t+1)$ple point τv.

(a) m even $=2m'$. The simplified abbildung-system; § 1.

$\mathfrak{C}^{m'+1}$ may degenerate into the line τ taken m' times, and a line through $\mathfrak{A}$; for this the limiting case $t=m'-1$.

The $(2t+2)$spread, locus of osculating R_{t+1} along τ^{t+1} is of order $(t+1)(m-2t)$. But the $(m-2t)$spread, locus of osculating R_{m-t-1} along $\tau^{m'-t}$ is also of order

$$(m'-t)(m-2m'-t-1)=(t+1)(m-2t).$$

For instance, $t=0$; the two-spread locus of lines R_1 (i. e., the original two-spread) is of order m; and, also, the m-spread locus of osculating R_{m-1} along $\tau^{m'}$ is of order m.

Thus, when m is even, the orders of the $(2t+2)$spread for $t=t_1$, $t=t_2$, are equal, if $t_1+t_2=m'-1$.

(b) m odd $=2m''+1$.

$t\leqq m$; since the curve $\mathfrak{C}^{m''+1}$ in the limiting case degenerates into the line τ taken $t+1=m''+1$ times.

For $t=m''$ the order of the $(2t+2)=2m''+2=(m+1)$spread is $(m''+1)(m-2m'')=m''+1$; i. e., through *every point* $\mathrm{R}_{m''+1}$ of R_{m+1} may be passed $m''+1$ R_m meeting the 2-spread in an $(m''+1)$ple line τ.

There is no symmetry analogous to that for m even.

4. *Curves on the two-spread.*

(b) m odd $=2m''+1$.

The abbildung-system, $\mathfrak{C}^{m''+1}$ having $\mathfrak{O}$ as m''-ple pt.

Let us denote the *unique* curve of order m'' in an m''-flat corresponding to $\mathfrak{O}$ by O; and the right lines of the spread by τ. A curve on the spread of order p, meeting the unique curve O in q points and every line τ in r points may be written $\mathrm{C}^p\,(\mathrm{O}^q\tau^r)$.

A curve on the abbildung plane $\mathfrak{C}^s\,(\mathfrak{O}^t)$ of the order s with a t-ple point at $\mathfrak{O}$ is met by a curve of the system $\mathfrak{C}^{m''+1}$ in $s(m''+1)-tm''$ points and by any line τ in $s-t$ points; therefore, it corresponds to a curve on the spread of order $s(m''+1)-tm''$, which meets the unique curve O in t points, and every line τ in $(s-t)$ points, say

$$\mathrm{C}^{s(m''+1)-tm''}\,(\mathrm{O}^t\tau^{s-t}).$$

So a curve $\mathfrak{C}^s\,(\mathfrak{O}^t\tau^{s-t})$ transforms into $\mathrm{C}^{s(m''+1)-tm''}\,(\mathrm{O}^t\tau^{s-t})$.

A curve $\mathrm{C}^p\,(\mathrm{O}^q)$, of order p, meeting the curve O in q pts., must have

$$p>q \text{ and } p\equiv q\ (\text{mod. } \overline{m''+1});\ \text{say } p=s(m''+1)-tm'',\quad q=t.$$

$s(m''+1)-tm''=p$. $(s\geqq t)$.

$s=1, t=1, p=1$. A line τ on $\mathfrak{R}_2$ corresponds to a line τ on the spread.

$s=s$, $t=s$, $p=s$. s lines τ correspond to s lines τ.

If $s>t$, $p\geqq m''+1$; which shows that the curve O of order m'' *is unique*, since it is the only curve of so low an order on the two-spread (the lines τ excepted).

Curves on the two-spread and in flats of less than $m+1=2m''+2$ dimensions are *full skew* curves. Such a curve is an R_m-intersection or a part of an R_m-intersection; therefore, its abbildung is a curve $\mathfrak{C}^s(\mathfrak{O}^t)$; any $m''+1-s$ lines τ belong to the supplementary system, of which the asyzygetic number is $m''+2-s$; therefore, $m''+2-s$ asyzygetic R_m meet in an $R_{m''+s}$ in which the curve $C^{s(m''+1)\;(s-1)m''-m''+}$ lies. A curve $C^{m''+s}$ in $R_{m''+s}$ is a full skew curve. (I; theorem C.)

The general plane curve $\mathfrak{C}^s$ ($t=0$; not through $\mathfrak{O}$) corresponds to a $C^{s(m''+1)}$ which does not meet the unique curve. The *plane* is a full skew 2-spread $S_{2,1,2}$ ($m=2m''+1=1$, $m''=0$). All the geometry of *plane* curves depending upon intersections and tangencies and the order of curves is immediately applicable to the *general* full skew two-spread of *odd* order $m=2m''+1$, the curves $C^{s(m''+1)}$ on the two-spread corresponding completely to the curves $\mathfrak{C}^s$ of the plane. A curve $C^{s(m''+1)-tm''}(O^t)$ meeting the unique curve O t times is a particular case of $C^{s(m''+1)}$, and in fact plays the same rôle as a $C^{s(m''+1)}$ having a t-ple point. A few examples are given.

There is a double infinity of curves $C^{m''+1}$; two meet in one point; one is determined by two points; they correspond to the lines $\mathfrak{C}^1$ of the plane. A line τ together with the unique curve O is a special case of a curve $C^{m''+1}$. Five points determine a curve $C^{2(m''+1)}$; which corresponds to a conic $\mathfrak{C}^2$ of the plane.

Pascal's theorem becomes:

If six points $P^1\,..\,P^6$ lie on a curve $C^{2(m''+1)}$ the three points of intersection of the two curves $C^{m''+1}$ joining P^1P^2, P^4P^5; P^3P^4, P^5P^6; P^6P^1, P^2P^3, respectively, lie on another curve $C^{m''+1}$.

To a curve $C^{s(m''+1)-tm''}$ $(O^t\tau^{s-t})$ there are $s(s-1)-t(t+1)=(s+t)(s-t-1)$ tangent lines τ, and $s(s-1)-t(t-1)=(s-t)(s+t-1)$ tangent curves $C^{m''+1}$ in a pencil through a point P.*

An m-spread of order P meets the 2-spread in a curve C^{mP} meeting the unique curve O $m''P$ times, and every line τ in P points; $\therefore C^{mP}\equiv C^{(2m''+1)P}(O^{m''P}\tau^P)$. $s=(m''+1)P$, $t=m''P$. To an m-spread of order P in $R_{2m''+2-m+1}$ there are $mP(P-1)$ tangent lines lying entirely on the $S_{2,m,m+1}$.

Two curves $C\,(O^t\tau^{s-t})$, $C\,(O^{t'}\tau^{s'-t'})$, meet in $ss'-tt'$ points; in particular, two curves $C\,(O^t\tau^{s-t})$ meet in s^2-t^2 points; one is determined by

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* These formulæ are similar to some given by Chasles, *Comptes Rendus*, 1861; cf. the following (a).

$\frac{1}{2}\{s(s+3)-t(t+1)\}$ points. Hence two curves $C^s(O^t\tau^{s-t})$ through $\frac{1}{2}\{s(s+3)-t(t+1)\}-1$ points determine a pencil of such curves through these and $\frac{1}{2}\{s(s-3)-t(t-1)\}+1$ additional points

(a) m even $=2m'$. The simplified abbildung-system, $\mathfrak{C}^{m'+1}$ having $\mathfrak{O}$ as m'-ple point and $\mathfrak{A}$ as an ordinary point.

Curves $\mathfrak{C}^{p+1}(\mathfrak{O}^p,\mathfrak{A}^1)$ of order $p+1$ having $\mathfrak{O}$ as p ple pt., through $\mathfrak{A}$, correspond to *full skew* curves of order $m'+p$; these are the only curves on the spread in a flat of less than $m+1$ dimensions. The proof is like that of (b) for m odd.

In particular:—A line $y_2-\tau y_1=0$ through $\mathfrak{O}$ corresponds to a line τ on the spread (§ 2); two lines τ do not meet. A line v through $\mathfrak{A}$ corresponds to a full skew curve v of order m' on the spread; two curves v do not meet. Through every point on the spread pass one line τ and one curve $C^{m'}$, v; a line τ and a curve v meet in one point.

$\mathfrak{O}$ corresponds to a curve O of order m', meeting every line τ.

$\mathfrak{A}$ corresponds to a line A of order m', meeting every curve v.

The curve O meets the line A in a point OA. (§ 2.)

In fact, the curve O, the line A, the point OA in no way differ from an ordinary curve v, line τ, point τv of the spread.

Observe that a curve $\mathfrak{C}^s(\mathfrak{O}^t\mathfrak{A}^u)$ of order s (having O a t-ple and A an u-ple point), corresponds to a curve $C^{(s-t)m'+(s-u)}(\tau^{s-t}v^{s-u})$ of order $(s-t)m'+(s-u)$ meeting every line τ in $s-t$ points and every curve v in $s-u$ points: it passes $s-t-u$ times through the point OA and meets the line A elsewhere in u points, the curve O elsewhere in t points; ($\therefore$ in all, it meets the line A in $s-t$ points, the curve O in $s-u$ points).

A curve $\mathfrak{C}^{s'=(s-t)+(s-u)}(\mathfrak{O}^{t'=(s-u)}\mathfrak{A}^{u'=(s-t)})$ with an $(s-t-u)$ple point at say $\mathfrak{Q}$ corresponds to a curve $C^{(s-t)m'+(s-u)}(\tau^{s-t}v^{s-u})$ with an $(s-t-u)$-ple point at Q. Thus the order and character of intersection with the lines τ and curves v and the $(s-t-u)$ple point are exactly the same. The asyzygetic numbers are equal; as shown by the following equality (where $v=s-t-u$, $s'=s+v=2s-t-u$, $t'=s-u$, $u'=s-t$),

$$\begin{aligned}&(s+1)(s+2)-t(t+1)-u(u+1)\\ &=(s'+1)(s'+2)-t'(t'+1)-u'(u'+1)-v(v+1)\end{aligned}$$

The statement above is justified, and it is therefore proper to consider only curves which have *no* especial relation to the point OA; i. e., in the abbildung-plane, only curves $\mathfrak{C}^s(\mathfrak{O}^t\mathfrak{A}^u)$ where $s=t+u$.

The spread is *ruled* with the lines τ (§2); and *also* with the curves v, $C^{m'}$. The curves v correspond to $y_3-vy_2=0$; the $(m'+2)$spread

$$\left\|\begin{matrix} X_{m'+2}, & X_{m'+3}, & X_{m'+4} & \ldots\ldots & X_{m+1} \\ X_{m'+3}, & X_{m'+4} & \ldots\ldots & X_{m+1}, & X_{m+2} \end{matrix}\right\| = 0$$

is cut in the curves υ by m'-flats, the intersection of corresponding R_m in the $m'+1$ projective pencils of m-flats R_m,

$$X_{m'+2} - \upsilon X_1 = 0, \quad X_{m'+3} - \upsilon X_2 = 0 \;\ldots\ldots\; X_{m+2} - \upsilon X_{m'+1} = 0.$$

A curve $\mathfrak{C}^{t+u}(\mathfrak{G}^t\mathfrak{J}^u)$ corresponds to a curve $C^{um'+t}(\tau^u\upsilon^t)$ of order $um'+t$ meeting each line τ in u points, each curve υ in t points.

Since the number of intersections of curves on the two-spread with the lines τ, the curves υ and with each other, and all intersection- and tangency-properties, depend only on the abbildung-curves, it is clear that there is a *complete correspondence* between the curves on a two-spread of even order $m=2m'$, and those on an ordinary hyperboloid or quadric ($m'=1$); the two systems of ruling curves, the lines τ and the curves υ of order m', answer to the two systems of generators on the quadric (only, in the latter case, the two systems being of the same order are indistinguishable). Hence many of Chasles' results (*Comptes Rendus*, liii, 1861) concerning "Propriétés générales des courbes gauches tracées sur l'hyperboloide" apply in this more general case; for example:

A curve $C^{um'+t}(\tau^u\upsilon^t)$ is determined by $tu+(t+u)$ points.

Two curves $C^{um'+t}(\tau^u\upsilon^t)$, $C^{u'm'+t'}(\tau^{u'}\upsilon^{t'})$, meet in $tu'+ut'$ points.

All curves $C^{um'+t}(\tau^u\upsilon^t)$ going through $tu+(t+u)-1$ fixed points form a pencil passing through $tu-(t+u)+1$ other fixed points; since any two meet in $2tu$ points.

To a curve $C^{um'+t}(\tau^u\upsilon^t)$ $2t(u-1)$ lines τ are tangent.
$2u(t-1)$ curves υ are tangent.
$2tu$ curves $C^{m'+1}(\tau'\upsilon')$ of a pencil through a pt. P are tangents.

These numbers are easily derived by considering the corresponding abbildung-curves.

The curve $C^{um'+t}(\tau^u\upsilon^t)$ corresponds to a curve $C^{u+t}(\tau^u\upsilon^t)$ on an ordinary quadric; on the quadric there is no distinction between the curves $C^{u+t}(\tau^u\upsilon^t)$, $C^{u+t}(\tau^t\upsilon^u)$; i. e., two curves of the same order $t+u$, which meet the generators of one system τ in ^u_t points, and those of the other system υ in ^t_u points. If, then, there is a theorem about curves of order $u_r m'+t_r$ ($r=1, 2, \ldots S'$) meeting the lines τ in u_r pts. and the curves υ in t_r pts., the *same* theorem will be true about corresponding curves of order $t_r m'+u_r$ meeting the lines τ in t_r pts. and the curves υ in u_r pts.

A curve $C^P(\tau^u\upsilon^t)$ must have $P=um'+t$.

An m-spread of order P intersects the 2-spread in a curve of order $mP = 2m'P$, meeting the lines τ in $u = P$ points, and the curves v in $t = m'P$ points; say $C^{2m'P}(\tau^P v^{m'P}) \equiv C^{Pm'+m'P}(\tau^P v^{m'P})$.

$$2t(u-1) = 2m'P(P-1) = mP(P-1)$$

lines τ are tangent to this curve.

There are $mP(P-1)$ lines τ lying entirely on the two-spread $S_{2,m,m+1}$ and tangent to an m-spread $S_{m,P,m+1}$ of order P.* (m *odd* or *even*; cf. § 4, b).

The curves on a full skew two-spread of even order, $m = 2m'$, then, have an exact correspondence with those on an ordinary quadric, $m = 2$; those on a full skew two-spread of odd order, $m = 2m'' + 1$, have almost as exact a correspondence with the curves on a plane, $m = 1$; the *unique* curve O is a singularity, but curves meeting it in t points play very much the same rôle on the two-spread as curves having a t-ple pt. at an ordinary point of the spread. These close correspondences with the hyperboloid and plane curves are the marked features of the theory of curves on full skew two-spreads.

III. SPREADS OF ODD ORDERS ON QUADRICS.

The known theorems, that a curve S_1 of odd order on an ordinary quadricone, cone-$Q_{2,3}$, passes an odd number of times through the vertex V, and that a general quadric 3-spread $Q_{3,4}$ contains no 2-spreads S_2 of odd order (cf. Clifford, *Mathematical Papers*, p. 64), may be extended.

$Q_{r,r+1}$ will denote a *general* quadric r-spread in R_{r+1}, and cone-$Q_{r,r+1}$ a quadric r-spread in R_{r+1} formed by joining a (general) $Q_{r-1,r}$ to a vertex-point V in R_{r+1}.

The section of $Q_{r,r+1}$ made by a tangent r-flat R_r is a cone-$Q_{r-1,r}$.

The section of a cone-$Q_{r,r+1}$ by an R_r through the vertex V is a cone-$Q_{r-1,r}$; but that by an arbitrary R_r is a (general) $Q_{r-1,r}$.

S_r will denote an r-spread.

A$_1$. The general $Q_{2m,2m+1}$ has on it no r-spread of odd order unless $r < m+1$.

A$_2$. The cone-$Q_{2m,2m+1}$ has on it no r-spread of odd order

A$_{21}$. unless $r < m+1$; while

A$_{22}$. m-spreads of $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ order pass an $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ number of times through the vertex V.

B$_1$. The general $Q_{2m-1,2m}$ has on it no r-spread of odd order unless $r < m$.

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* Chasles gives this for the case $m = 2$.

B_2. The cone-$Q_{2m-1,2m}$ has on it no r-spread of odd order unless

B_{21}. $r<m+1$; while

B_{22}. m-spreads of $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$order pass an $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$number of times through the vertex V.

Let B_1 hold in R_{2n}; then will the theorems A hold in R_{2n+1}.

A_1. A_{21}. Take arbitrary R_{2n} section of $\left.\begin{array}{r} Q_{2n,2n+1} \\ \text{cone-}Q_{2n,2n+1} \end{array}\right\}$ containing an r-spread of odd order; there results a (general) $\left.\begin{array}{l} Q_{2n-1,2n} \\ Q_{2n-1,2n} \end{array}\right\}$ with an $(r-1)$spread of odd order on it; hence, by B_1, $r-1<n$; or $r<n+1$; proving A_1, A_{21}.

A_{22}. Notice that the projection in R_{2m+1} from a pt. P upon an R_{2m} of an r-spread of order s passing t times through P is an r-spread of order $s-t$, i. e., of *odd* order, unless $s-t$ is even; i. e., unless the r-spread of $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ order passes an $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ number of times through P.

Take arbitrary R_{2n} section of cone-$Q_{2n,2n+1}$ containing an n-spread S_n; it is a $Q_{2n-1,2n}$ which contains the projection through P of S_n; if B_1 holds, the preceding consideration shows that A_{22} must also.

Hence, if the propositions hold for R_{2n} (i. e., the B), they do for R_{2n+1} (i. e. the A).

If the A ($m_A=n$) hold for R_{2n+1}, the B ($m_B=n+1$) will hold for R_{2n+2}.

B_1. The $Q_{2n+1,2n+2}$ contains an r-spread S_r of odd order; the tangent R_{2n+1} at a pt. V (not on the r-spread S_r), cuts the quadric in a cone-$Q_{2n,2n+1}$, containing an $(r-1)$spread of odd order not passing through the vertex V; hence, by A_{21},

$$r-1<n,\ r<n+1,\ r<m_B, \text{ proving } B_1.$$

B_2. An R_{2n+1}-section through the vertex V of the cone-$Q_{2n+1,2n+2}$ shows the dependence of B_{21}, B_{22} on the truth of A_{21}, A_{22}.

But A_2 holds for R_3. A curve of $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ order in an ordinary quadricone, cone-$Q_{2,3}$, passes through the vertex V an $\left.\begin{smallmatrix}\text{odd}\\ \text{even}\end{smallmatrix}\right\}$ number of times, because its projection through V upon a plane R_2 is (a conic) of *even* order.

From A_2 for R_3 follow at once the B for R_4, and thus the general propositions as enunciated for R_{2m}, R_{2m+1}.

IV. Flats on Quadrics.

Prof. Cayley, "On the Superlines of a Quadric Surface in 5-dimensional space" (*Quart. J. M.*, 1872–3, t. xii, p. 176) gives an analytical proof of the proposition, suggested by an evident theorem in *line-geometry;*—(using Clifford's expressions): On a quadric 4-spread $Q_{4,5}$ there are two triply infinite systems of planes $R_{2,a}$, $R_{2,b}$; two planes of the same system meet in a point; two of opposite systems in general do not intersect at all, if they do, it is in a line.

There is a similar theorem for quadrics in all flats of odd dimensions.

1. *On a quadric* 2m-*spread* $Q_{2m,2m+1}$ *in* (2m+1)*flat* R_{2m+1} *there are two* $\frac{1}{2}$m(m+1)*ply infinite systems of* m-*flats* R_m.*

If this holds for R_{2m-1}, then it will hold for R_{2m+1}. For R_1 ($m=1$) this is the double system of generators R_1 on a quadric surface.

Project upon a fixed r_{2m} from a pt. V on the $Q_{2m,2m+1}$. The tangent R'_{2m} at V cuts $Q_{2m,2m+1}$ in a cone-$Q_{2m-1,2m}$, with vertex at V, and it cuts r_{2m} in a fixed r'_{2m-1}, which contains the projection through V of the cone-$Q_{2m-1,2m}$ i. e., a fixed $q'_{2(m-1),2m-1}$.

An R_m on the $Q_{2m,2m+1}$ $\left.\begin{matrix}\text{not passing through V}\\ \text{passing through V}\end{matrix}\right\}$ meets the tangent R'_{2m} at V in $\left.\begin{matrix}R_{m-1}\\ R_m\dagger\end{matrix}\right\}$ and is projected into an $\begin{matrix}r_m\\ r_{m-1}\end{matrix}$ $\left.\begin{matrix}\text{having an } r_{m-1}\\ \text{lying completely}\end{matrix}\right\}$ on the fixed $q'_{2m-2,2m-1}$ in r'_{2m-1}.‡

The converse is also true.§

The $q'_{2(m-1),2m-1}$ is supposed to have on it two $\frac{1}{2}(m-1)m$-ply infinite systems of $(m-1)$flats r_{m-1}. In r_{2m} two r_m meet in a point; hence, there are ∞^m r_m through a fixed r_{m-1}, i. e., one joining the fixed r_{m-1} to every pt. of the ∞^m pts. of a random fixed r_m. These r_m correspond to R_m on the $Q_{2m,2m+1}$.

Therefore, on the $Q_{2m,2m+1}$, there are two $\infty^m . \infty^{\frac{(m-1)m}{2}} = \infty^{\frac{m(m+1)}{2}}$ $=\frac{1}{2}m(m+1)$ply infinite system of m-flats R_m.

2. *The intersections of the m-flats.*

(*k*) *m* odd.

Two R_m of the same system ($R_{m,a}$, $R_{m,a}$) in general do not intersect, but in special cases they may intersect in R_1, or R_3, or . . . or R_{m-2}.

Two of opposite systems ($R_{m,a}$, $R_{m,b}$) in general intersect in a point, but may intersect in R_2, or R_4, or . . . or R_{m-1}.

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* There is on the quadric no R_s if $s > m$; (III, A_1).

† If an R_m passes through V, it lies completely in the tangent R'_{2m} at V.

‡ Thus the intersection of an r_m (the projection of an R_m) with the fixed r'_{2m-1} lies completely on the fixed q'; and likewise two r_m and the fixed r'_{2m-1} intersect on the fixed q'.

§ A quadric m-spread passing through V would project into an m-flat r_m having a quadric $(m-2)$spread on q'.

(l) m even.

Two R_m of opposite systems in general do not intersect, but may intersect in R_1, or R , or . . . or R_{m-1}.

Two R_m of the same system in general intersect in a point, but may intersect in an R_0, or R_1, or . . . or R_{m-2}.

This may be expressed ($R_0 \equiv$ a point, $0 \equiv$ no intersection), beginning with the *most infrequent* cases:

Two R_m of $\begin{matrix}\text{the same}\\ \text{opposite}\end{matrix}\Big\}$ system intersect in

$$\left.\begin{matrix} R_{m-2}, \text{ or } R_{m-4}, \text{ or } & \ldots & \text{or } R_0 & , & 0 \\ R_{m-1}, \text{ or } R_{m-3}, \text{ or } & \ldots & \text{or } \ \ 0 & , & R_0 \end{matrix}\right\}$$

(1). A few considerations to be used in the proof are given here.

In r_{2m} two r_m meet in a pt. r_0; if in another pt., then in a line r_1; if two r_m have an r_s common, and also another pt., then they have an r_{s+1} in common.

Two r_m intersecting on q' in r_s may intersect in another point; and thus in r_{s+1}; but they do not intersect in *two* other asyzygetic points, for then the intersection with the fixed r'_{2m-1} would not lie entirely on the q'. (Cf. foot-note ‡, §1.)

If two r_m intersect only in an r_s lying on the q', the two corresponding R_m meet the R_{s+1} joining this common r_s to the fixed point V in two R_s (which were both projected into the common r_s) which in the R_{s+1} intersect in an R_{s-1}. As a special case, if two r_m intersect in a *point* r_0 on q', the two corresponding R_m [intersect the line R_1, joining r_0 to V, in two points R_0 and] do not intersect.

(2) m even. The r_{m-1} on q' intersect according to (k), $m-1$ being odd.

On q' $r_{m-1,a}$ and $r_{m-1,c}$ in general do not intersect, but may intersect in r_1, r_3 . . . . or r_{m-3}. Two r_m through them must intersect in a point, at least. Hence, in general, m being even, two R_m of the same system intersect in a point. In the particular cases, if the two r_m intersect entirely on q', the two R_m intersect in R_0, R_2 . . . or R_{m-1}; but if the two r_m intersect also in a point not on q', the two R_m intersect in R_2, R_4 . . . or R_{m-3}.

On q' $r_{m-1,a}$ and $r_{m-1,b}$ intersect in a point r_0; but may intersect in r_2, r_4 . . . or r_{m-2}. Two r_m through them would in general intersect in no external point; hence, in general, m being even, two R_m of opposite systems do not intersect. In the particular cases, if the two r_m intersect entirely on q', the two R_m intersect in R_1, R_3 . . . or R_{m-3}; but if the r_m intersect also in a point not on q', the two R_m intersect in R_1, R_3 . . . or R_{m-1}.

Thus, the (k) holding for $m-1$ odd, the (l) hold for m even.

(3) m odd. By similar considerations it is shown that, the (l) holding for m even, the (k) hold for $m+1$ odd. But the (k) hold for $m=1$ on the quadric Q_2 in R_3 (as might be shown immediately by the projection from V on a plane); and, therefore, (k) and (l) are true in general.

3. One m-flat of each system passes through every R_{m-1} of the Q_{2m}. The R_{m-1} projects into an r^{m-1} having an r_{m-2} on q', through which (suppose) there pass an $r_{m-1,a}$ and an $r_{m-1,b}$; the original r_{m-1} and the $r_{m-1,b}$, intersecting in r_{m-2}, determine an r_m the projection of an $R_{m,a}$ through the R_{m-1}. This is true on the Q_2; therefore, in general.

Every pt. in R_{2m+1} has a polar R_{2m} with reference to the Q_{2m}; the polar R_{2m} of every pt. in an R_s passes through a certain R_{2m-s}, which is called the polar of the R_s. (Clifford.)

If the s-flat R_s lie on the Q_{2m}, the polar R_{2m} of every point in it, i. e., the tangent R_{2m} at that point, passes through the s-flat itself; hence the polar P_{2m-s} must include the original s flat R_s.

The m-flats R_m are *self-polar.*

The $R_{m,a}$ and $R_{m,b}$ through an R_{m-1} taken together lie in and determine the polar R_{m+1} of the R_{m-1}.

More generally, two R_m intersecting in an R_s lie in and determine the polar R_{2m-s} of the R_s.

III.—ON KNOTS, WITH A CENSUS FOR ORDER TEN. By C. N. LITTLE, LINCOLN, NEB.

1. GAUSS in 1833[*] called attention to the importance of the study of the ways in which cords might be linked. Nothing, however, appears to have been written upon the subject until in 1847 Listing published his *Vorstudien zur Topologie.*[†] In this he briefly but in a masterly way touched upon the subject of knots, established some of the fundamental propositions, and proposed a notation which, as slightly modified by Prof. Tait, furnishes the point of view for the present paper. In a communication[‡] to Prof. Tait in 1877, Listing points out the fragmentary character of his own contribution to the subject, and says that the type-symbol used by him is "nichts weiter als ein derartiger Fingerzeig."

It is to Professor Tait, however, that the greater part of our present knowledge of the subject is due. He, independently of Listing, obtained the fundamental propositions and found the knots and their forms for orders from three to seven inclusive.[§]

In 1884 Kirkman[‖] published the forms of knots of orders eight and nine, and immediately Tait, making use of Kirkman's work, extended his census of knots to these orders.[¶]

2. Professor Tait has shown that any closed plane curve of n crossings divides its plane into $n+2$ compartments; that these compartments are in two groups; that, at the crossings, like compartments are vertically opposite. We shall call these compartments of the plane *parts.* A part is represented by the number equal to the number of double points on its perimeter. The sum of the numbers representing the parts of either group is $2n$, that is, these numbers together constitute a partition of $2n$. The partitions for the two groups together make up Listing's *type-symbol.* As it can lead to

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[*] "Eine Hauptaufgabe aus dem Grenzgebiet der *Geometria Situs* und der *Geometria Magnitudinis* wird die sein, die Umschlingungen zweier geschlossener oder unendlicher Linien zu zahlen."—Werke. Gottingen. 1867, vol. v, p. 605.

[†] Gottingen Studien, 1847. I have been able to see only Tait's apparently full abstract in Proc. Roy. Soc. Edin., vol. ix, pp. 306–309.

[‡] Proc. Roy. Soc. Edin., vol. ix, p. 316.

[§] On Knots, Trans. Roy. Soc. Edin., xxviii, 145–191, 1876–77

[‖] Trans. Roy. Soc. Edin., xxxii, 281–309.

[¶] Trans. Roy. Soc. Edin., xxxii, 327–342 no ambiguity we shall also call the number representing a compartment a *part*, and either group of compartments a *partition*.

Since every closed plane curve of n crossings, having double points only, may be read alternately over and under at the crossings, every such curve which gives parts, none greater than n or less than 2, may be taken as a projection of a reduced knot of n crossings. We call such curves *knot-forms* or briefly, *forms*, and regard two forms as distinct if they do not have the same parts similarly arranged.

The first part of the problem is to find all the different knot forms of any order.

Since the same knot may be transformed so as to be projected into more than one knot-form, the second part of the problem is, from the complete series of knot-forms of n crossings to find all the different n-fold knots. Knots exist for which the law of over and under does not hold; these are not considered in the present paper.

3. It is unnecessary to do more than allude to two very distinct and very ingenious methods devised and used, the one by Tait and the other by Kirkman, for the solution of the first part of this problem. We may perhaps infer from Professor Tait's opinion* that "a full study of 10-fold and 11-fold knottiness seems to be relegated to the somewhat distant future," that they were more laborious than proves to be necessary.

4. A third method, based on Listing's type-symbol, is thus described by Professor Tait at page 168 of his first memoir.

"Write all the partitions of $2n$, in which no one shall be greater than n and no one less than 2. Join each of these sets of numbers into a group, so that each number has as many lines terminating in it as it contains units. Then join the middle points of these lines (which must not intersect one another), by a continuous line which *intersects* itself at these middle points and there only. When this can be done we have the projection of a *knot*. When more continuous lines than one are required we have the projection of a linkage."

On page 160 of the same memoir, he says, speaking of this method: "But we can never be quite sure that we get *all* possible results by a semi-tentative process of this kind. And we have to try an immensely greater number of partitions than there are knots, as the great majority give links of greater or less complexity."

It seems possible however, with the help of some simple theorems to make the "Partition Method" exhaustive, and wholly to do away with the drawing of links.

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* In 1884. Trans. R. S. E., xxxii, 328

5. An inspection of form A*a* of Plate I will make clear some terms already introduced and others that we shall now require. Regarding the curve A*a* as alone in a plane, it divides it into twelve *parts*, two 9-gons, two 3-gons and eight 2-gons. The external 3-gon or *amplexum* differs in no way from the other parts. Of these twelve parts, two 9-gons and one 2-gon form one group—the *leading* partition; the two 3-gons and seven 2-gons form the other group—the *subordinate* partition. The terms leading and subordinate are relative merely, but that partition will be taken as leading which has the smaller number of parts. The *type-symbol* for A*a* is $\left\{\begin{matrix} 9^2 2 \\ 3^2 2^7 \end{matrix}\right.$.

6. The double points common to the perimeters of two parts of the same partition will be called *bonds* of those parts, and the parts are said to be bound by these bonds. It is well known that a type-symbol does not determine a form. For this, it is necessary to know the numbers of bonds between the several parts of either partition, together with the arrangement of these parts.

In general the parts of a given partition may be bound in more than one way giving forms that may be projections of either links or knots. Each set of numbers of bonds of the several parts of the given partition is a *clutch* of that partition.

The *class* p of a partition is the number ot parts in it. The class of a form is the class of its leading partition. The *order* of any partition is equal to n and is the same as the order of all knot-forms derivable from it. The *deficiency* $\varkappa$ of a partition is its order minus its greatest part.

7. Let the parts of $2n$ be A, B, C, . . . P arranged in order of magnitude, and the numbers of bonds of each part be respectively $\alpha, \beta, \gamma, \ldots \pi$. Let the number of bonds common to any two parts as A and B be (AB). Then

$$\left.\begin{array}{l} (AB)+(AC)+ \ldots\ldots\ldots (AP)=\alpha \\ (AB)+(BC)+ \ldots\ldots\ldots (BP)=\beta \\ (AC)+(BC)+ \ldots\ldots\ldots (CP)=\gamma \\ \quad\cdot \qquad \cdot \qquad \cdot \qquad \cdot \qquad \cdot \qquad \cdot \\ (AP)+(BP)+ \ldots\ldots\ldots (OP)=\pi \end{array}\right\} (a)$$

or n equations with $\frac{1}{2}n(n-1)$ unknown quantities which can have only positive integral values. The possible solutions of (a) will evidently give all the clutches for this partition.

8. I. THEOREM.—If a part be solely bound to a second part, or if any q parts ($q \not< p-2$) be bound mutually in any way and all free bonds of these parts go to a single part, then this portion of the form constitutes a separate knot (unless there be linkage) and the string concerned in it may be drawn tight without affecting the remainder of the knot-form. Such knots are not considered as belonging to order n.

In particular a 2-gon so bound throws out from consideration a clutch.

9. II. THEOREM.—No knot-form of the nth order has as leading partition one whose class exceeds $\kappa+2$.

Adding equations (a) above, dividing by two, and subtracting the first and any other, say the second, we find

$$\begin{aligned}-(AB)+(CD)+(CE)+\;.\;.\;.\;(CP)+\;.\;.\;.\;(OP) &= n-\alpha-\beta,\\ &= \kappa-\beta.\end{aligned}$$

Therefore, $(AB) \not< \beta-\kappa$.

In a similar way

$$\begin{aligned}(AC) &\not< \gamma-\kappa\\ (AD) &\not< \delta-\kappa\\ .\;\;.\;\;&.\;\;.\;\;.\\ (AP) &\not< \pi-\kappa\end{aligned}$$

Adding $(AB)+(AC)+\;.\;.\;.\;(AP) \not< \beta+\gamma+\;.\;.\;.\;\pi-(p-1)\kappa$

$$\begin{aligned}&\not< n+\kappa-(p-1)\kappa\\ &\not< n-(p-2)\kappa,\end{aligned}$$

we have then the two conditions

$$\left.\begin{aligned}(AB)+(AC)+\;.\;.\;.\;(AP) &\not< n-(p-2)\kappa\\ &= n-\kappa.\end{aligned}\right\}\;(b)$$

Now suppose, if possible, $p=\kappa+3$

$$\begin{aligned}(AB)+(AC)+\;.\;.\;.\;(AP)=n-\kappa \qquad &= n-\kappa\\ \not< n-(\kappa+1)\kappa\;&\not< n-\kappa-\kappa^2.\end{aligned}$$

To the minimum values of (AB), (AC), etc., (that is, to $\beta-\kappa$, $\gamma-\kappa$, . . .) must be added κ^2 in all, and to no one more than $\kappa-1$, by I. The $\kappa+2$ smallest parts of κ square are evidently $(\kappa-1)^4$ $(\kappa-2)^{\kappa-2}$. By adding these $\kappa+2$ parts in any way to the minimum values, a clutch will be given in which each of four parts will have a single bond not going to A, and each of $\kappa-2$ parts will have two. A part of the latter kind cannot have its two free bonds carried to a second part of the same kind, by I. If two parts be joined by a single bond there will be left two free bonds. Ultimately it will be necessary to join two parts of the first kind to a combination of parts having but two free bonds, and I will apply. If any of the $\kappa+2$ parts of κ^2 be diminished by s then will s parts of $2n$ be added to those of the first kind, and however the free bonds may be arranged, ultimately the same result as before will be reached. Therefore p cannot equal $n+3$ and still give knot-forms.

Much less can p be greater than $n+3$.

10. A given clutch of a leading partition does not uniquely determine a form. The following proposition however holds.

III. Theorem.—All or none of the forms determined by any given clutch of a partition are knot-forms of the order considered.

For, all forms to be had from any clutch of a given partition may be obtained by taking all the possible different changes (consistent with the given clutch) of relative position of the various parts. But these can all be effected by successive interchanges of the connections of two parts, whether such connections are direct (by a single bond), by a 2-gon, by a 3-gon, or are more complicated. We may therefore confine the attention to a definite portion of the knot and keep the remainder fixed. Let A and B, (Fig. 1, Plate I), be two parts connected as shown. Two strings, or two parts of a single string, are involved. If there were more all but two would be closed. Let the ends of these strings, or parts of a single string, leave the portion of the form under consideration at a and c on the perimeter of A, and b and d on that of B. Cut at these points and call the ends a and a', b and b', c and c', d and d'; a, b, c and d remain fixed. Now revolve through 180° about the axis AB, and join the free ends.

Before the change there may be three cases. The strings may be $\left\{\begin{matrix} c\,d \\ a\,b \end{matrix}\right.$ $\left\{\begin{matrix} b\,d \\ a\,c \end{matrix}\right.$ or $\left\{\begin{matrix} b\,c \\ a\,d \end{matrix}\right.$ After the change a' is joined to c, and c' to a; b' to d, and d' to b.

$$\begin{array}{lcl} \left\{\begin{matrix} c\,d \\ a\,b \end{matrix}\right. & \text{become} & \left\{\begin{matrix} c\,a'\,b'\,d \\ a\,c'\,d'\,b \end{matrix}\right. \\ \left\{\begin{matrix} b\,d \\ a\,c \end{matrix}\right. & \text{"} & \left\{\begin{matrix} b\,d'\,b'\,d \\ a\,c'\,a'\,c \end{matrix}\right. \\ \left\{\begin{matrix} b\,c \\ a\,d \end{matrix}\right. & \text{"} & \left\{\begin{matrix} b\,d'\,a'\,c \\ a\,c'\,b'\,d \end{matrix}\right. \end{array}$$

Therefore coming up to this part of the knot on any string, we must leave on the same string before and after the change. If then the form was a knot before the change it will be one after, and if a link before it will be a link after.

11. *Coils.*—A succession of n 2-gons constitutes an *n-coil*, which may be open or closed. Since at the 3rd or $2n+1$st crossing of a coil the strings have the relative position of the first crossing, if the coil be closed by carrying around the ends to the beginning and

joining them so as to preserve the law of over and under one string will be formed. While if from the $2n$th crossing the strings are carried around, that string over at the 1st is under at the $2n$th and, on joining, there will be two strings.

Hence, as is well known, ${(2n+1)^2 \atop (2)^{2n+1}}$ is always a knot, while ${(2n)^2 \atop (2)^{n}}$ is always a link.

12. For the purpose of distinguishing between clutches giving knots and those giving links, it follows from Theorem III that we may take the direct bonds between any two parts together, and these form open coils of the subordinate partition; and further it is evident from § 11 that any odd open coil (even number of bonds), may be dropped, and any even coil (odd number of bonds) may be replaced by a single bond. If the resulting clutch gives link, so would the original. If the resulting clutch be still too complex for easy recognition of its character, the clutch of the subordinate partition of the resulting form may perhaps be still farther reduced in the same way. If the clutches of lower orders were at hand they also could be used for settling the question.

13. We have the following theorems for throwing out clutches unproductive of knot forms.

IV. Theorem.—If a part be joined to other parts in every case by an even number of bonds, there is linkage. For, the string about this part is closed by Section 12.

V. Theorem.—If two parts are connected by two 2-gons (of the same partition with the parts) there is linking. For they may be put in succession by Theorem III. When this is done there is a 4-gon of the negative partition bound to two other parts in each case by two bonds, and IV applies.

VI. Theorem.—An odd part joined to one part by an odd number of bonds and to other parts in every case by an even number of bonds may be dropped; for, by Theorem III it becomes a loop with a single crossing, and this can have no effect on the question of linking.

In particular a 3-gon joined by one bond to one part and by two bonds to a second part may be dropped.

If two odd parts are joined by an odd number of bonds, and are joined to other parts in every case by an even number of bonds there is linkage.

In particular two 3-gons so joined throw out the clutch.

VII. Theorem.—If two 3-gons, C and D, are themselves joined directly and are joined to A and B in each case by a single bond there is linkage.

For, HCM (fig. 2, Plate I) under we will say at C is over at H and M. LCN is then over at C and under at L and N. LHN is over at L, under at H, and over at N. Its continuation is therefore NML which is over at N, under at M, and over at L.

14. Class III, Order n.—In this class we have

$$(AB)+(AC)+(BC)=n$$

and an unique solution of equations (α), § 9. Therefore $(BC)=\varkappa$, $(AB)=\beta-\varkappa$ and $(AC)=\gamma-\varkappa$. If two or three of these quantities be even, the clutch to which they belong will give a linkage, by Theorem IV. In other cases by § 12 there is a knot.

Suppose n to be odd and α even; then $\varkappa$ is odd, and β and γ are both odd, or both even. In the first case the clutch gives a link, in the second a knot.

Suppose n to be odd, and α odd; then $\varkappa$ is even, and of β and γ one must be odd and the other even. The clutch gives a link.

Suppose n to be even and α even; then $\varkappa$ is even and β, γ are both odd or both even. In the first case a knot, in the second a link.

Suppose n to be even and α odd; then $\varkappa$ is odd, and of β and γ one must be odd and one even, and there is a knot. This proves the following:

VIII. Theorem.—In odd orders only partitions of $2n$ into three even parts, give knots, while in even orders, only these partitions give links.

15. Class IV, Order n. Here

$$\begin{aligned}-(AB)+(CD)&=\varkappa-\beta\\ -(AC)+(BD)&=\varkappa-\gamma\\ -(AD)+(BC)&=\varkappa-\delta\\ (AB)+(AC)+(AD)&\begin{smallmatrix}=n-\varkappa\\ \not<n-2\varkappa\end{smallmatrix}\end{aligned}$$

The minimum values of (AB), (AC), (AD), (BC), (BD), (CD), are respectively $\beta-\varkappa$, $\gamma-\varkappa$, $\delta-\varkappa$, 0, 0, 0. To get every clutch we must add in every possible way to the minimum values of (AB), (AC), (AD), all the partitions of $\varkappa$ into not more than three parts, none greater than $\varkappa-1$. But evidently we must increase the minimum values of any quantity of the second set (BC), (BD), (CD), by the same number that we increase the corresponding quantity of the first set. The following scheme which considers in detail every possible case, expresses clearly the propositions for determining whether clutches of partitions of this class furnish knots or links. Let e or o indicate whether a number be even or odd.

n,	κ	Partition	Add $\kappa=$	$\beta-\kappa$ $\gamma-\kappa$ $\delta-\kappa$	AB AC AD	BC BD CD	Form	Props or Sect used
e	e	e e e e	e e e	e e e	e e e	e e e	Link	IV.
			e o o		e o o	o o o	"	§12 V
		o o o o	e e e	o o o	o o o	o e e	"	IV
			e o o		o e e	o o e	"	IV
			o e o		o o e	o e o	Knot	§12 VI §11
	o	o o o o	o o o	e e e	o o o	o o o	Link	§12, VII
			o e o		o e e	e o o	"	§12, VI
		o o e e	o o o	e o o	o e e	o o o	Knot	§12, VI, 11
			o e e		o o o	e e o	"	§12, VI, 11
			e o e		o e o	e o o	Link	IV
o	e	o o e e	e e e	o o e	o e e	e e e	"	IV
			e o o		o o o	o o o	"	§12, V
			o e o		e e o	o e o	Knot	§12
	e	o o o o	e e e	o o o	o o o	o e o	"	§12,
			e o o		o e e	o o o	"	§12
	o	e e e e	o o o	o o o	e e e	o o o	Link	IV
			o e e		e o o	e o o	"	IV
		e e o o	o o o	o e e	e o o	o o o	"	§12, V.
			o e e		e e e	e o o	"	IV
			e o e		o o e	e o o	Knot	§12

The first line of this scheme says that when n is even and κ even, and $2n$ divided into four even parts, the minimum values of (AB), (AC), (AD), will be even, and that if a partition of κ into three even parts be added to $\beta-\kappa$, $\gamma-\kappa$, $\delta-\kappa$ the numbers constituting the clutch will be even, and the form a link by Prop. IV.

One of the propositions proved in this scheme is worthy of separate enunciation.

IX. THEOREM.—In even orders partitions of $2n$ into four even or four odd parts give link-forms only. In odd orders partitions into four even parts give link-forms only.

16. X. THEOREM.—In all orders partitions of six even parts give link-forms only.

For, an even number may be divided into five parts, of which four, two or none shall be odd. After the application of §12 the only parts to be found in the leading partition will be 4-gons and 2-gons. Every form under consideration will be reduced so as to have as leading partition one of the following, with a clutch of which every number is 1.

$$4^6, \quad 4^5 2, \quad 4^4 2^2, \quad 4^3 2^3, \quad 4^2 2^4, \quad 4 2^5, \quad 2^6.$$

In the lower orders $4^3 2^3$, $4^2 2^4$, 42^5, and 2^6 have been found to give no knots.

If the form given by 4^6 be drawn it is found in any particular case to consist of four closed curves, and therefore by Theorem III is always a link-form. On drawing $4^4 2^2$ it also proves to be a linkage.

The partition $4^5 2$ with the given clutch can not exist in a plane. This happens in two cases in order 10. On drawing the forms in such cases additional crossings will be found to be necessary. It is therefore true that in all orders, partitions of six even parts give link-forms only.

A synthetic proof of this theorem is possible.

17. Instead of continuing the consideration of the general subject we shall now illustrate the method of determining the knot-forms of any order by a particular consideration of order 10.

The number of partitions of 20 into parts none greater than 10 or less than 2 is 107.* These, arranged in dictionary order, are given in full in Table I.

Of these the single partition of Class II gives a link, § 11; the thirty-seven marked II are cut out by Theorem II. In Class III, Theorem VIII throws out $8^2 4$ and 86^2; in Class IV, Theorem IX the eight partitions marked IX; and in Class X, Theorem X the three marked X. The partitions remaining in Classes III to VI inclusive are alone taken as leading partitions; for, all remaining partitions appear in every possible way as subordinate partitions (§ 2), and can, therefore, furnish no additional knot-forms.

We have then to tabulate the clutches of those partitions still remaining in Classes III to VI. We at once write down Tables II and III in which are omitted all clutches that are thrown out by Sections 14 and 15. In Class V, a table with headings as shown in Table IV is used. We take for illustration the first partition, $7^2 2^3$. Here $\varkappa=3$, and $\beta-\varkappa$, $\gamma-\varkappa$, $\delta-\varkappa$, $\varepsilon-\varkappa$ are 4, -1, -1, -1. In this class we must add in every possible way to the minimum values of (AB), (AC), (AD), (AE) the partitions of $2\varkappa$ into four parts, or fewer, none greater than $\varkappa-1$. The only partitions of 6 meeting these conditions are 2^3 and $2^2 1^2$, which may be added in seven different ways to 4, -1, -1, -1, giving the different clutches of the table. Thus adding 1, 2, 2, 1, to $\beta-\varkappa$, $\gamma-\varkappa$, $\delta-\varkappa$, $\varepsilon-\varkappa$ we have (AB), (AC), (AD), (AE), equal to 5, 1, 1, 0, respectively. Subtracting (AB) from β, etc.

$$\begin{aligned}
\Sigma\mathrm{B} &= (\mathrm{BC}) + (\mathrm{BD}) + (\mathrm{BE}) = 2\\
\Sigma\mathrm{C} &= (\mathrm{BC}) + (\mathrm{CD}) + (\mathrm{CE}) = 1\\
\Sigma\mathrm{D} &= (\mathrm{BD}) + (\mathrm{CD}) + (\mathrm{DE}) = 1\\
\Sigma\mathrm{E} &= (\mathrm{BE}) + (\mathrm{CE}) + (\mathrm{DE}) = 2.
\end{aligned}$$

These quantities are put in the columns headed ΣB, ΣC, ΣD, ΣE, and all the possible solutions of this set of equations here as throughout the work are written down by inspection. The rest of this table is made out in the same way. In Table V we must, in every possible way, add the partitions of $3n$ into four parts or fewer, none greater than $n-1$. In the general case we add the partitions of $(p-3)n$ into $p-1$ parts none greater than $n-1$. In the complete Table IV there are 400 clutches, and in Table V 1000. It has not been thought necessary to publish more than a sample of either table.

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* See Tait, Trans. Roy. Soc. Edin., vol. xxxii, p. 342.

18. Having completed the tables of clutches we next cast out in Tables IV and V all clutches unproductive of knot-forms. The theorems already established are sufficient for this purpose. The routine followed will be readily understood from considering its use in the samples given of Tables IV and V.

Partition $7^2 2'$: in clutch (1) A and B are connected by two 2-gons, and V applies; (4) becomes (3) by interchanging D and E; (7) becomes (6) by interchanging C and D; and in (2) and (5) we have a 2-gon solely bound to a single part, and I applies.

Partition $6^2 4 2^2$: clutch (1) is thrown out by IV since a 6-gon B is joined to A by four bonds and to C by two; Theorem IV casts out (4) because of C, (14) because of C, (17) because of B, and (20) because of A; in (2), (3), (8), (9), (11), (12), (15) and (18) a 2-gon is solely joined to a single part, and I applies; in (5) B and C form a combination solely bound to A, and I applies; I throws out also (10) where D and E are bound together and to C, and (13) where the same parts are bound to B; interchanging D and E (7) becomes (6); interchanging A and B (16) becomes (7).

Partition $5^3 32$: I throws out (2), (3), (6), (8), (14), (16), (19), (20), (26), (27), (30), (31), (33), (37) and (38); by interchanges of parts

$(5)\equiv(4)$, $(17)\equiv(5)$, $(24)\equiv(9)$, $(32)\equiv(11)$,
$(7)\equiv(1)$, $(18)\equiv(3)$, $(25)\equiv(4)$, $(34)\equiv(12)$,
$(13)\equiv(10)$, $(21)\equiv(10)$, $(28)\equiv(23)$, $(35)\equiv(1)$,
$(15)\equiv(12)$, $(22)\equiv(21)$, $(29)\equiv(23)$, and $(36)\equiv(9)$.

In (4) D is dropped by VI, and B thus is changed to a 4-gon; then the application of § 12 leaves the two 3-gons A and C joined by two 2-gons, E and the still farther reduced B; V, therefore, shows that the clutch gives links only. In (9), drop C by VI and B becomes a 2-gon and A a 3-gon; the two 3-gons A and D are connected by the two 2-gons E and the reduced B; therefore the clutch gives links, by V. In (11) drop C by VI and the two 3-gons A and B are connected by the two 2-gons E and the reduced D; V applies.

In Table V, clutch No. (1) of 53^5 is thrown out by I. In (2) the two 3-gons E and F are joined together by a single bond and to A in each case by two bonds, and VI applies; or we might use I. Theorem VI throws out (4), since the two 3-gons B and F are joined to each other by a single bond and to other parts in each case by two bonds. In (5), drop F, by VI; B becomes a 2-gon joining D and E, and they are 3-gons connecting A and C. An obvious extension of Theorem VII throws out the clutch. Since in (7) B and C are joined together and to A, I applies.

This process is continued until all clutches which do not give knot-forms have been thrown out, as well as those clutches which are repetitions of clutches previously given. The samples given of Tables IV and V constitute about one-twentieth of the complete tables.

19. We are now ready to draw the forms which the remaining clutches furnish.

Definition.—The number of *Circular arrangements* of *n* things is the number of distinct ways in which *n* things can be arranged in a circle, the order whether direct or retrograde being of no consequence.

For illustration of the general method we consider in detail the productive clutches of partition $6^2 42^2$. In clutch (6) the two 6-gons are connected, by three bonds, by a 2 gon, and by a 4-gon which is connected with one 6-gon by two bonds and with the other by a 2-gon and one bond. There will be as many forms from this clutch as there are circular arrangements of a a a b (cd), where c and d may not be separated. These are

a a a b (cd)
a a a b (dc)
a a b a (cd)
a a b a (dc).

The four forms C^2c_1, C^2c_2, C^2c_3, C^2c_4, can at once be drawn. See Plate V, Knot LXIV.

In (19), the only other clutch of this partition that affords forms, a 6-gon is connected with a 4-gon directly, by a 2-gon, and by a 6-gon which is connected with the 4-gon by a single bond and by a 2-gon. There are two circular arrangements of a b (cd), namely a b (cd) and b a (cd); these furnish the two forms C^2d_1, C^2d_2. Knot XXX, Plate III. In practice the operations described in this section and in the preceding are performed simultaneously.

20. In Class VI every productive partition is used as a leading

partition. But since the subordinate partitions here belong to the same class, every form, with certain exceptions, will be found twice; this affords a check on the completeness of this part of the work. The exceptions are the *amphicheiral* knot-forms. These have the same partitions similarly connected both as leading and as subordinate partitions. These therefore appear but once.

In the partition 53^6, which was given as the shortest possible illustration of Table V, clutch (3) furnishes a single form D^2s_2, which already had been obtained under 54^232^2; clutch (6) gives two which had been obtained, Du_1 under 643^22^2 and D^2x_1 under 54^232^2. Clutch (8) gives the only new knot-form from this partition, viz: the amphicheiral D'o.

From the clutches of Classes III, IV, V and VI 364 ten-fold knot forms are obtained.

21. *The Derivation of Knots from Knot-Forms.*—Prof. Tait has not described the methods which he used in his derivation of the knots of lower orders from the knot-forms. In 1884 he says :* "the treatment to which I have subjected Kirkman's collection of forms, in order to group together mere varieties or transformations of one special form, is undoubtedly still more tentative in its nature; and thus, though I have grouped together many widely different forms, I cannot be *absolutely* certain that all those groups are essentially different from one another."

If a ten-fold knot be placed upon a plane in such a way as to have but ten crossings the eye will project it upon the plane in a form which will be found among the 364 above obtained. If the knot gives more than one form it will be possible to obtain any other of its forms by one or more turnings over of restricted portions of the knot while the remainder is held fixed. Now the string cannot issue from the portion of the knot that is turned at more than four points, for in that case the turning would introduce consecutive overs, and one or more additional crossings; the portion of the knot that is turned must therefore be wholly between two parts of the given knot form and in turning it we untwist two of the strings at one point and twist two at another, the result being simply to change the position of a single bond from one end of the connection to the other. The class of the form is therefore not changed, and all the forms of any knot belong to the same class.

22. Moreover in order 10 and lower orders all the forms coming from any clutch are obtained by changing the position of single

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* Trans. Roy. Soc. Edin., vol. xxxii, p. 327.

bonds in the connections of pairs of parts. Therefore in these orders all forms from any clutch are forms of the same knot. The subordinate partitions of these forms are then to be examined and all forms added which are obtained from them by changing the positions of connections of their parts, retaining the given clutch of the subordinate partition. These forms in turn are treated in the same way, but it will usually happen that no new form of the knot is obtained and the complete determination of the knot in all of its forms is finished.

23. We take for illustration Knot I of Class IV, shewn on Plate I. $B\alpha_1$ becomes $B\alpha_2$ by twisting about a vertical axis the 2 gon connecting the 8-gon and 7-gon. The first crossing below is opened and the strings above are crossed, the rest of the knot remaining fixed. Twisting the 2-gon again $B\alpha_3$ is obtained and nothing new is gotten by further changes of the forms. Since the negative partition in every case consists of two parts joined by three symmetrical connections, which have only one circular arrangement, there are no other forms of Knot I.

24. The knots of Class III, order n, are unique; since three things have but one circular arrangement.

25. The knots of Classes III, IV, V will be found with their forms grouped together in Plates I–V. On Plates V–VII are figured the forms of Class VI grouped as they come from the clutches, except that no form is repeated. The knots of this class will be found in Table VI. Every knot-form is the projection of two knots, one of which is the perverted image of the other, and consequently each group of knot-forms belongs to two knots which are in general different. If a series of knot-forms contains any amphicheiral form then it will also contain the perversion of every form of the series not amphicheiral. The series consists of the forms of one knot and not of two.

26. In order 10 I find, counting a knot and its irreconcilable perversion as two:

Class.	Forms.	Knots.	Knots.
III,	6	12	6
IV,	25	30	15
V,	200	128	64
VI,	133	64	39
Totals,	364	234	124

In lower orders Professor Tait has found:

Orders.	Forms.	Knots.	Knots.
3	1	2	1
4	1	1	1
5	2	4	2
6	3	5	3
7	10	14	7
8	27	31	18
9	100	82	41

The fourth column contains the numbers as they are given by Tait, the perversions of knots not being counted.

In so long a labor as is involved in making such a census the opportunities for error are many. Any errors or omissions that may be found in the census are to be attributed to the writer rather than to the method, which is simple and direct.

TABLE I.—*Partitions of* 20.

	CLASS II.		CL. IV.—Contin'd		CL. V.—Contin'd.		CLASS VI.		CL. VII.—Conti'd
	10^2	IX.	8^22^2	II.	862^3	II.	102^5	II.	642^5
	CLASS III.		8732		8532^2	II.	932^4		63^22^4
II.	1082	IX.	8642		84^22^2	II.	842^4		5^22^5
	1073		863^2		843^22		83^22^3		5432^4
	1064		85^22		83^4	II.	752^4		53^32^3
	105^2		8543		7^22^3		7432^3		4^32^4
	9^22	IX.	84^3		7632^2		73^32^2		$4^23^22^3$
	983		7^242		7542^2	X.	6^22^4		43^42^2
	974	IX.	7^23^2		753^22		6532^3		3^62
	965		7652		74^232	X.	64^22^3		
VIII.	8^24		7643		743^3		643^22^2		CLASS VIII.
	875	IX.	75^23		6^242^2		63^42	II.	62^7
VIII.	86^2		754^2		6^23^22		5^242^3	II.	532^6
	7^26	IX.	6^32		65^22^2		$5^23^22^2$		4^22^6
	CLASS IV.		6^253		65432		54^232^2		43^22^5
II.	1062^2	IX.	6^24^2		653^3		543^32		3^42^4
	10532		65^24		64^32		53^5		
	104^22	IX.	5^4		64^23^2	X.	4^42^2		CLASS XI.
	1043^2		CLASS V.		5^332		4^33^22	II.	42^8
II.	972^2	II.	1042^3		5^24^22		4^23^4		3^22^7
	9632		103^22^2		5^243^2				
	9542	II.	952^3		54^33		CLASS VII.		CLASS X.
	953^2		9432^2		4^5	II.	82^6		
	94^23		93^32			II.	732^5		2^{10}

TABLE II.—*Clutches for* $p=3$.

Partition.	(AB)	(AC)	(BC)		Partition.	(AB)	(AC)	(BC)		Partition.	(AB)	(AC)	(BC)	
9^22	8	1	1	Aa	974	6	3	1	Ac	875	5	3	2	Ad
983	7	2	1	Ab	965	5	4	1	Ae	7^26	4	3	3	Af

TABLE III.—*Clutches for $p=4$.*

Partition.	$\beta-\kappa$ $\gamma-\kappa$ $\delta-\kappa$	(AB) (AC) (AD)	(BC) (BD) (CD)	(ΣB) (ΣC) (ΣD) *	
8732	5 1 0	5 2 1	1 1 0	2 1 1	Ba$_1$, Ba$_2$, Ba$_3$.
$\kappa=2$ add 1, 1		6 1 1	1 0 1	1 2 1	Bb.
8633	4 1 1	5 1 2	1 0 1	1 2 1	Bc.
8552	3 3 0	3 4 1	1 1 0	2 1 1	Bd$_1$, Bd$_2$.
8543	3 2 1	3 3 2	1 1 0	2 1 1	Be$_1$, Be$_2$.
		4 3 1	0 1 1	1 1 2	Bf.
7742	4 1 −1	5 1 1	2 0 1	2 3 1	Bg.
$\kappa=3$ add 2, 1		5 2 0	1 1 1	2 2 2	Same as Bg A:B.†
1, 1, 1					
7652	3 2 −1	3 3 1	2 1 0	3 2 1	Bh$_1$, Bh$_2$.
		4 3 0	1 1 1	2 2 2	Bi.
7643	3 1 0	5 1 1	1 0 2	1 3 2	Bj.
		3 3 1	1 2 0	3 1 2	Bk$_1$, Bk$_2$.
		4 2 1	1 1 1	2 2 2	Bl.
7544	2 1 1	3 3 1	0 2 1	2 1 3	Bm.
		3 2 2	1 1 1	2 2 2	Bn.
6653	2 1 −1	3 1 2	3 0 1	3 4 1	Bo.
$\kappa=4$ add		3 3 0	1 2 1	3 2 3	Same as Bo A:B.
3, 1		3 2 1	2 1 1	3 3 2	Bp.
2, 1, 1					
2, 2					
6554	1 1 0	1 2 3	3 1 0	4 3 1	Br.
		3 2 1	1 1 2	2 3 3	Bs.
		4 1 1	1 0 3	1 4 3	Bq.

* $\Sigma B=\beta-AB=BC+BD$, etc. † A:B signifies that A and B are to be interchanged.

TABLE IV.—*Clutches for $p=5$.*

Partition.	Add	$\beta-\kappa$ $\gamma-\kappa$ $\delta-\kappa$ $\varepsilon-\kappa$	(ΣB) (ΣC) (ΣD) (ΣE)	(AB) (AC) (AD) (AE)	(BC) (BD) (BE)	(CD) (CE) (DE)	No.
$7^2 2^3$	0222	4 −1 −1 −1	3 −1 −1 −1	4 1 1 1	1 1 1	0 0 0	(1) V.
$\kappa=3$ add	2211		1 1 2 2	6 1 0 0	1 0 0	0 0 2	(2) I.
Parti'n of 2κ					0 1 0	0 1 1	(3) Ca.
222					0 0 1	1 0 1	(4) Same as (3) D:E.
2211	1221		2 1 1 2	5 1 1 0	0 0 2	1 0 0	(5) I.
					1 0 1	0 0 1	(6) Cb$_1$, Cb$_2$, Cb$_3$.
					0 1 1	0 1 0	(7) Same as (6) C:D.
*	*	*	*	*	*	*	* * *
$6^2 4 2^2$	2033	2 0 −2 −2	2 4 1 1	4 0 1 1	2 0 0	1 1 0	(1) IV.
$\kappa=4$ add	3023		1 4 2 1	5 0 0 1	1 0 0	2 1 0	(2) I.
332	0323		4 1 2 1	2 3 0 1	1 2 1	0 0 0	(3) I.
3311	0233		4 2 1 1	2 2 1 1	2 1 1	0 0 0	(4) IV.
3221	1133		3 3 1 1	3 1 1 1	3 0 0	0 0 1	(5) I.
2222					2 1 0	0 1 0	(6) $C^2c_1C^2c_2C^2c_3C_2c_4$.
					2 0 1	1 0 0	(7) ≡ (6) D:E.
	3122		1 3 2 2	5 1 0 0	0 0 1	2 1 0	(8) I
					0 1 0	1 2 0	(9) I.
					1 0 0	1 1 1	(10) I.
	1322		3 1 2 2	3 3 0 0	0 1 2	1 0 0	(11) I.
					0 2 1	0 1 0	(12) I.
					1 1 1	0 0 1	(13) I.
	1223		3 2 2 1	3 2 0 1	2 1 0	0 0 1	(14) IV.
					1 2 0	0 1 0	(15) I.
					1 1 1	1 0 0	(16) ≡ (7) A:B.
	2123		2 3 2 1	4 1 0 1	2 0 0	1 0 1	(17) IV.
					1 0 1	2 0 0	(18) I.
					1 1 0	1 1 0	(19) C^2d_1, C^2d_2.
	2222			4 2 0 0			(20) IV.
*	*	*	*	*	*	*	* * *

TABLE IV.—*Clutches for p 5 (continued).*

Partition	Add	$\beta-\kappa$	$\gamma-\kappa$	$\delta-\lambda$	$\varepsilon-\lambda$	(ΣB)	(ΣC)	(ΣD)	(ΣE)	(AB)	(AC)	(AD)	(AE)	(BC)	(BD)	(BE)	(CD)	(CE)	(DE)	No.
5^332	0214	0	0	−2	−3	5	3	1	1	0	2	2	1	3	1	1	0	0	0	(1) C^3y.
$\kappa=5$ add	0424					5	1	3	1	0	4	0	1	1	3	1	0	0	0	(2) I.
442	1144					4	4	1	1	1	1	2	1	1	0	0	0	0	1	(3) I ABCA
4411														3	1	0	0	1	0	(4) VI, §12, V.
433														3	0	1	1	0	0	(5) (1) B:C
4321	0433					5	1	2	2	0	4	1	0	1	2	2	0	0	0	(6) I
4222	0331					5	2	2	1	0	3	1	1	2	2	1	0	0	0	(7) (1) A:B
3331	0343					5	2	1	2	0	3	2	0	2	1	2	0	0	0	(8) I.
3322	1234					4	3	2	1	1	2	1	1	3	1	0	0	0	1	(9) VI, §12, V.
														2	2	0	0	1	0	(10) C^4z_1, C^3z_2, C^4z_1.
														2	1	1	1	0	0	(11) VI, V.
	1243					4	3	1	2	1	2	2	0	3	0	1	0	0	1	(12) C^4a
														2	1	1	0	1	0	(13) — (10) A:B.
														2	0	2	1	0	0	(14) I.
	1324					4	2	3	1	1	3	0	1	2	2	0	0	0	1	(15) — (12) A:B
														1	3	0	0	1	0	(16) I.
														1	2	1	1	0	0	(17) — (5) A:B.
	1423					4	1	3	2	1	4	0	0	1	2	1	0	0	1	(18) — (3) A:B.
														0	3	1	0	1	0	(19) I
														0	2	2	1	0	0	(20) I.
	2224					3	3	3	1	2	2	0	1	1	1	1	2	0	0	(21) —(10) A:B & B:C
														1	2	0	1	1	0	(22) —(21) B.C.
														2	1	0	1	0	1	(23) C^4b.
	1333					4	2	2	2	1	3	1	0	2	1	1	0	0	1	(24) (9) A B.
														1	2	1	0	1	0	(25) — (1) A:B
														1	1	2	1	0	0	(26) I.
	2233					3	3	2	2	2	2	1	0	3	0	0	0	0	2	(27) I.
														2	1	0	0	1	1	(28) — (23) B.C A:B
														2	0	1	1	0	1	(29) — (23) A:B.
														1	2	0	0	2	0	(30) I.
														1	0	2	2	0	0	(31) I.
														1	1	1	1	1	0	(32) — (11) B:CA A:B
	2323					3	2	3	2	2	3	0	0	0	3	0	0	2	0	(33) I.
														1	2	0	0	1	1	(34) (12) B:A & B:C
														0	2	1	1	1	0	(35) —(1) A:B & B:C
														1	1	1	1	0	1	(36) (9) A:B & B:C
														2	1	0	0	0	2	(37) I.
														0	1	2	2	0	0	(38) I.
*	*	*				*				*				*			*			* * *

TABLE V – *Clutches for p=*

Partition	Add	$\beta-\kappa$	$\gamma-\kappa$	$\delta-\lambda$	$\varepsilon-\kappa$	$\phi-\lambda$	(ΣB)	(ΣC)	(ΣD)	(ΣE)	(ΣF)	(AB)	(AC)	(AD)	(AE)	(AF)	(BC)	(BD)	(BE)	(BF)	(CD)	(CE)	(CF)	(DE)	(DF)	(EF)	
*	*	*							*					*				*				*			*		* *
53^5	22344	−2	−2	−2	−2	−2	3	3	2	1	1	0	0	1	2	2	3	0	0	0	0	0	0	1	1	0	(1) I.
$\kappa=5$																	2	1	0	0	1	0	0	0	0	1	(2) VI.
																	1	1	1	0	1	0	1	0	0	0	(3) Knot D s_2 of 54^232^2.
	23334						3	2	2	2	1	0	1	1	1	2	2	0	0	1	0	0	0	2	0	0	(4) VI.
																	0	1	1	1	1	1	0	0	0	0	(5) VII.
																	0	2	1	0	0	1	1	0	0	0	(6) Knot Du_1 of 643^22^2, knot D^2x_1 of 54^232^2
	33333						2	2	2	2	2	1	1	1	1	1	2	0	0	0	0	0	0	1	1	1	(7) I.
																	1	1	0	0	0	1	0	0	1	1	(8) Knot D^4o Amph.
*	*	*							*					*				*				*			*		* * *

TABLE VI.—*Knots of Class* VI.

Knot.	No.	Forms.
I	6	Da_1, Da_2, Da_3, Da_4. Da_5, Da_6.
II	1	Db.
III	4	Dc_1, Dc_2, Dc_3, Dc_4.
IV	4	De_1, De_2 De_3, De_4.
V	2	Df_1, Df_2.
VI	4	Dk_1 Amph, Dk_2‖,* D^2j Amph.
VII	1	Dl Amph.
VIII	3	Dm, D^2t_1, D^2t_2.
IX	16	Dn_1 Amph, Dn_2‖, Dn_3‖. Dn_4‖, D^3i_1‖. D^3i_2 Amph. D^3h_1‖, D^3h_2‖, D^3h_3 Amph, D^3s Amph.
X	12	Du_1, Du_2, Du_3, Du_4, Du_5, D^2x_1, D^2x_2, D^2x_3, D^2x_4, D^2x_5, Do_1, Do_2.†
XI	12	Dp_1, Dp_2, Dp_3, Dp_4, Dp_5, Dp_6, D^2e_1, D^2e_2, D^2e_3. D^2e_4. D^2e_5, D^2e_6.
XII	9	Dq_1, Dq_2, Dq_3. D^2f_1, D^2f_2. D^2f_3, D^3c, D^2u, D^2h
XIII	6	Dr_1, Dr_2, Dr_3, D^2q_1, D^2q_2, D^2q_3.
XIV	3	Ds, D^2c_1, D^2c_2.
XV	2	Dt, D^2d.
XVI	9	Dv_1, Dv_2, Dy_1, Dy_2, Dy_3. D^2g_1. D^2g_2, D^2y, D^3j.
XVII	9	Dw_1‖, Dw_2 Amph, Dw_3‖, D^2l_1 Amph, D^2l_2‖, D^3l Amph.
XVIII	6	Dx_1, Dx_2, Dx_3, D^3a_1, D^3a_2, D^2a.
XIX	4	Dz_1 Amph, Dz_2‖, D^3b Amph.
XX	1	D^2b Amph.
XXI	3	D^2i_1, D^2i_2, D^2i_3.
XXII	1	D^2k.
XXIII	1	D^2m.
XXIV	1	D^2n.
XXV	3	D^2p_1‖, D^2p_2 Amph.
XXVI	3	D^2r_1 Amph, D^2r_2.
XXVII	3	D^2s_1, D^2s_2. D^2s_3.
XXVIII	2	Dv_1, Dv_2.
XXIX	2	D^2w_1, D^2w_2.
XXX	2	D^2z_1, D^2z_2.
XXXI	1	D^3d Amph.
XXXII	1	D^3e Amph.
XXXIII	4	D^3f Amph, D^3f_2‖, D^3q Amph.
XXXIV	1	D^3g.
XXXV	1	D^3k.
XXXVI	1	D^3m Amph.
XXXVII	1	D^3o Amph.
XXXVIII	1	D^3p Amph.
XXXIX	1	D^3r.

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* The symbol ‖ indicates that a form and its perversion are both included.

† The subordinate partition of Do_2, Plate V. should be 643^22^2.

IV.—THE AMYLOLYTIC ACTION OF DIASTASE OF MALT, AS MODIFIED BY VARIOUS CONDITIONS; STUDIED QUANTITATIVELY. BY R. H. CHITTENDEN AND GEO. W. CUMMINS, PH.B.

THE close relationship existing between the diastase of malt and the amylolytic ferment of saliva has led us to make a careful study of the conditions favorable to the action of the former, in the hope of obtaining confirmation of previous results obtained with the salivary ferment.* The widespread use, moreover, of malt extracts as therapeutic agents lends to the work in question a practical interest, which in no wise detracts from its value.

Falk† has recorded that the diastase of malt loses its amylolytic power under the influence of dilute acid, similar to the ferment of saliva; that it is made inactive by gastric juice and that the retarding influence of a dilute acid (say 0·0135 per cent.) on its amylolytic power is diminished by the presence of peptone, owing to the probable formation of a peptone-acid compound. Falk, moreover, states that the retarding action of hydrochloric acid is due to destruction of the ferment, since on neutralization of the acid, amylolytic power is not restored. Kjeldahl‡ has recorded that dilute acids in very small quantity retard the amylolytic action of diastase; if, however, smaller, minimum quantities of acid are added the amylolytic power of diastase is increased. The same investigator§ has also noticed a like accelerating action of very small quantities of acid on invertin. Basnitz‖ has found that the presence of carbonic acid invariably increases the amylolytic power of diastase. Detmer¶ has recorded the same fact and in addition, that small quantities of citric acid as well as of phosphoric and hydrochloric acid increase the diastatic power of malt. Larger quantities of these acids render the malt extract inactive. Detmer has also found that the presence of a very slight alkaline reaction diminishes the amylolytic power of the ferment. Brown and Heron** state that a malt extract neutralized with barium hydroxide has its amylolytic power somewhat weakened; thus implying that the ferment acts more vigorously in the naturally acid extract than in a neutral fluid. The same investigators found that making the extract faintly alkaline with sodium carbonate also diminished somewhat the activity of the ferment, while sodium hydroxide completely stopped the action of the ferment. A like result was also obtained on the addition of 0·05 per cent. salicylic acid.

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* Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 343.

† Virchow's Archiv, vol. lxxxiv, p. 119.

‡ Jahresbericht für Thierchemie, 1879, p. 382.

§ Jahresbericht fur Thierchemie, 1881, p. 449.

‖ Berichte der deutsch chem. Gesell., vol. xi, p. 1443.

¶ Zeitschrift fur physiol. chemie, vol. vii, p. 2.

** Liebig's Annalen der Chemie, vol. cxcix, pp. 236–238.

Such are the recorded statements bearing on this question. Few quantitative results are given, and the influence of proteid matter, aside from its connection with dilute acid, has not been considered.

Method employed.

A fresh malt extract was prepared for each series of experiments, since the fluid tends rapidly to become acid, owing to the development of schizomycetes. The extract was prepared from coarsely ground malted barley, by simply extracting it with water at 40° C. for two to three hours (5 grams barley to 100 c. c. water), then filtering, neutralizing and diluting to 500 c. c.

Owing to the great difficulty of obtaining perfectly neutral starch, that used in the present work was prepared from potatoes, thoroughly washed and dried, making a starch perfectly neutral to the most delicate test papers. The volume of each digestive mixture in the various experiments was 100 c. c., containing 1 gram of starch previously boiled with a portion of the water, a definite quantity of the malt extract and a given amount of acid, alkali, or proteid matter, except in the control, which was naturally free from the latter. In determining amylolytic power the digestive mixtures were warmed at 40° C. for thirty minutes, after which further ferment action was stopped by boiling the fluid. The extent of amylolytic action was then ascertained by determining in one-fourth of the fluid, made up to 100 c. c., the amount of reducing bodies by means of Allihn's* gravimetric method; the reducing bodies being then calculated, for the sake of convenience, to dextrose, from which in turn, was calculated the percentage of starch converted.

Influence of sodium carbonate on the amylolytic action of diastase.

Previous experiments† with saliva have shown that the percentage of alkaline carbonate which absolutely or to a certain extent hinders its amylolytic action can be designated only for a definite mixture and not in a general sense, owing to variations in the amount of proteid matter present and doubtless also in part to increase or decrease in the amount of ferment.

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* Zeitschrift fur Analytische Chemie, vol. xxii, p. 448. † Chittenden and Smith.

It became necessary, therefore, at first, to ascertain something regarding the relative amylolytic action of the malt extract, which contains some proteid matter. Three quantities of malt extract were employed, which by thirty minutes warming at 40° C. with 1 gram of starch in the manner already described, gave the following results:

Malt extract.	Wt Cu in ¼	Total amount reducing bodies	Starch converted
10 c. c.	0·1192 gram	0·2428 gram	21·85 per cent
15	0·1489	0·3038	27·31
25	0·1543	0·3150	28·35

It is interesting to note here that, as in the case of the amylolytic ferment of saliva, there is no quantitative relation between the amount of ferment and the extent of amylolytic action; it is only when the ferment solution is greatly diluted that amylolytic action can be taken as a definite measure of the amount of ferment present. Kjeldahl* has likewise studied the influence of the quantity of diastase upon the amount of sugar formed under given conditions and he came to the conclusion that the formation of sugar was proportional to the amount of ferment only up to a certain point; beyond which, increase in the amount of ferment was not accompanied by proportional increase in the formation of sugar.

Preliminary experiments showed us that the ferment of malt is very susceptible to the action of sodium carbonate; the addition of even 0·025 gram of the alkaline carbonate to 15 c. c. of perfectly neutral malt extract, with subsequent dilution to 100 c. c. allowed no diastatic action whatever. Following are two series of experiments illustrating the action of different percentages of sodium carbonate† on the ferment under different degrees of dilution.

a with 15 c. c. of the standard malt extract

Na$_2$Co$_3$.	Wt. Cu in ¼	Total amount reducing bod:	Starch converted
0	0·1371 gram	0·2791 g	25·11 per cent
0·0005 per cent	0·1318	0·2684	24·15
0·0010	0·1274	0·2596	23·36
0·0020	0·0544	0·1124	10·11
0·0050	0·0197	0·0434	3·90
0·0080	0·0134	0·0312	2·80
0·0100	0·0135	0·0314	2·82
0·0125	0·0065	0·0148	1·33
0·0250	0		

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* Jahresbericht fur Thierchemie, 1879, 381.

† The standard solutions of sodium carbonate employed in these experiments were made from the chemically pure anhydrous salt.

b. with 30 c. c. of the standard malt extract.

Na_2Co_3.	Wt Cu in %.	Total amount reducing bodies	Starch con'
0	0·1650 gram.	0·3372 gram.	30·34
0·001 per cent.	0·1578	0·3224	29·01
0·003	0·1165	0·2986	26·87
0·005	0·1147	0·2334	21·00
0·010	0·0380	0·0796	7·16
0·025	0·0238	0·0516	4·64
0·050	0		

It is evident from these results that the amylolytic power of diastase, like that of ptyaline, is diminished in proportion as the percentage of alkaline carbonate is increased. Moreover, it would appear by comparison with results previously obtained* that diastase is far more susceptible to the action of sodium carbonate than ptyaline, and also that dilution of the malt extract does not so materially affect the retarding action of the different percentages of alkaline carbonate as in the case of saliva. Both of these results, however, may be due either to the presence of a larger amount of proteid matter in the saliva or to the presence of a larger proportion of ferment, or in fact, to both. It is noticeable in both series of experiments that the amylolytic power of the ferment after gradually diminishing appears to receive a sudden check, which in the larger amount of malt extract is produced by just double the percentage of carbonate requisite with the smaller amount of malt. In this way the effect of dilution is apparent and shows moreover that the exact influence of a given percentage of the alkaline carbonate can be designated only for a definite mixture.

Destructive action of sodium carbonate on diastase.

In order to ascertain how far the retarding action of sodium carbonate is due to destruction of the ferment, the following experiment was tried. Six mixtures were made as follows:

	1	2	3	4	5	6
Malt extract . .	30 c.c.	30 c.c.	30 c.c	30 c.c.	30 c.c.	30 c.c.
Na_2Co_3 sol.	0 “	1·25 “ 0·1%	2·5 “ 0·1%	2·5 “ 0·5%	5 “ 0·5%	10 “ 0·5%
H_2O	20 “	18·75 “	17·5 “	17·5 “	15 “	10 “
	50	50	50	50	50	50
Per cent. Na_2Co_3	0	0·0025	0·005	0·025	0·05	0·1

These were warmed at 40° C. for 1 hour, then neutralizing and equalizing mixtures were added as follows:

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* Chittenden and Smith.

	1	2	3	4	5	6
0·1 per cent. HCl	0	0·12 c. c.	0 85 c. c.	4 25 c. c.	8 5 c. c	16 95 c. c.
0·5 " Na_2Co_3 }*	10 c. c.	9·75	9·5	7 5	5·0	0
0·1 " HCl	16·95	16·55	16·1	12·7	8 45	0

The six solutions were now exactly alike; neutral to test papers and contained the same amounts of diastase and sodium chloride. They had, however, been exposed to the action of the above percentages of sodium carbonate for 1 hour at 40° C. Their amylolytic power was now determined in the usual manner (action on 1 gram of starch in a total dilution of 100 c. c.) with the following results:

No.	Wt. Cu in ¼	Total amount reducing bodies.	Starch converted
1	0·1739 gram.	0·3558 gram.	32 02 per cent.
2	0·1737	0·3554	31·97
3	0·1745	0·3570	32 13
4	0·0341	0·0722	6·49
5	0 0319	0·0678	6·10
6	0·0281	0·0602	5·41

No destructive action is apparent until 0·025 per cent. sodium carbonate is reached; warming the malt extract with 0·005 per cent. sodium carbonate causes no destruction whatever, while with 0·025 per cent. destruction is very great. The amount of malt extract (30 c. c.) experimented with, being the same as was used in determining the influence of alkaline carbonate on the amylolytic power of the ferment, the two series of results are directly comparable and show plainly that the retarding action of small percentages, in the present case up to 0·005 per cent., is due to simple retardation without destruction of the ferment. Beyond this point, however, as in the presence of 0·025 per cent. the greatly diminished amylolytic action is due to destruction of the ferment. Hence it would appear that in the case of the diastase of malt the destructive action of sodium carbonate is out of all proportion to its retarding action. This apparent difference, however, between diastase and the ptyaline of saliva is due, as we shall show later on, to the comparatively small amount of proteid matter in the malt extract. Saliva very greatly diluted, so that the percentage of proteid matter is reduced to a minimum, shows similar results.

Influence of neutral peptone on the amylolytic action of diastase.

It was demonstrated some time since,† that the presence of neutral peptone tends to increase the amylolytic action of neutral saliva.

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* The two equalizing mixtures were united before being added to the main solutions.

† Chittenden and Ely, Amer. Chem. Jour., vol. iv, 107.

Langley and Eves* have confirmed this statement, although they do not believe in the theory of a direct stimulation of the ferment, advanced by one of us. We find now that neutral peptone added to a neutral solution of malt diastase, similarly increases its amylolytic action; the increase being even greater than noticed in the case of neutral saliva. Two series of experiments were tried with the following results; the peptone used being made perfectly neutral with a dilute solution of sodium carbonate.

a. with 15 c.c. of the standard malt extract.

Peptone.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1140 gram.	0·2320 gram.	20·88 per cent.
0·1 per cent.	0·1545	0·3154	28·38
0·2	0·1512	0·3081	27·75
0·3	0·1494	0·3048	27·43
0·5	0·1457	0·2970	26·73
1·0	0·1427	0·2910	26·19

b. with 30 c. c. of the standard malt extract.

0	0·1785 gram.	0·3654 gram.	32·88 per cent.
0·1 per cent.	0·1847	0·3772	33·94
0·3	0·1912	0·3916	35·24

Peptone causes increased amylolytic action throughout; with 15 c. c. of malt extract, the smallest amount of peptone gives the greatest acceleration, which slowly diminishes as the percentage of peptone is increased; with 30 c. c. of malt extract, however, acceleration, which is much less than in the preceding series, increases with the increase in peptone. It is hard to find any reason for this acceleration in amylolytic action, other than a direct stimulation of the ferment.

Influence of sodium carbonate on the amylolytic action of diastase in the presence of proteid matter.

Proteid matter tends to prevent the retarding action of sodium carbonate on this ferment, as in the case of the salivary ferment. Thus, the addition of neutral peptone to a malt extract allows vigorous amylolytic action to take place in the presence of percentages of sodium carbonate, which alone would completely destroy the ferment. The following experiments, using 15 c. c. of the standard malt extract in each instance, illustrate the influence of peptone on the action of sodium carbonate.

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* Journal of Physiology, vol. iv, No. 1.

With 0·5 per cent neutral peptone.

Na_2CO_3.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted
0	0·1388 gram.	0·2828 gram.	25·15 per cent
0·001 per cent.	0·1443	0·2912	26·17
0 002	0·1431	0·2918	26·27
0·003	0·1485	0·3030	27·27
0·004	0·1404	0·2860	25·71
0 005	0·1406	0·2861	25·77
0·010	0·1317	0·2682	24·13

It is thus seen that the presence of 0·5 per cent. of neutral peptone entirely prevents the retarding action of the several percentages of sodium carbonate, except in the last experiment of the series where slight retardation is apparent. It is to be remembered here that even 0·005 per cent. of sodium carbonate alone, almost completely stops the action of the ferment. What at first sight appears to be strange in this last series of experiments, is that the first three percentages of sodium carbonate cause a gradual increase in amylolytic action over that of the neutral fluid plus like percentage of peptone. The explanation of this, however, is quite simple. In studying the influence of neutral peptone on the action of the ferment (15 c. c. malt) it was found that the greatest acceleration of amylolytic action was obtained with the smallest percentage of peptone, and moreover that ferment action diminished in proportion as the peptone was increased. Now peptone undoubtedly prevents the action of sodium carbonate on the ferment by combining with it, forming an alkaline carbonate-proteid compound, possessed of but little retarding action; hence in the above experiment the first action of the smallest percentages of sodium carbonate is to diminish the amount of free peptone, thus causing slight acceleration; further on, however, the increased amount of alkaline-proteid body formed, counteracts the accelerating influence of the free peptone, when gradual retardation commences; finally, increase in the percentage of sodium carbonate leads to the presence of free sodium carbonate, when amylolytic action comes to a sudden standstill. This point being reached, increasing the percentage of peptone prevents the stoppage of ferment action. This is well illustrated by the following series of experiments, using larger percentages of both peptone and sodium carbonate, but 15 c. c. of the malt extract, as before.

Neutral peptone	Na_2CO_3	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted
1·0 per cent.	0	0·2078 gram	0·4268 gram	38·41 per cent.
1·0	0·010 per cent.	0·1583	0·3234	29·10
1·0	0·025	0·1529	0·3122	27·09
1·0	0·050	0·1351	0·2751	24·78
1·0	0·100	0·0086	0·0209	1·88
2·0	0·100	0·1341	0·2730	24·57

Here, as before, the retarding action of sodium carbonate is held in check by the peptone, although there is slight retardation due to the alkaline-proteid body formed. Finally, the percentage of sodium carbonate being increased beyond the necessary proportion of peptone, there is a sudden cessation of ferment action. Increasing the amount of peptone, however, prevents this retarding action; evidently the alkaline-proteid body is without much effect on the ferment, only slowly diminishing its amylolytic power.

Influence of acid proteids on the amylolytic action of diastase.

Falk has noticed that peptone prevents to a certain extent, the retarding action of dilute acid on this ferment; no quantitative results, however, have been recorded, nor has any attempt been made to ascertain whether said action is due to simple retardation, or destruction of the ferment, or both. The action of acids, whether free or combined with proteid matter, on the diastase of malt is particularly important, in view of the rapid passage of the ferment into the stomach when taken in therapeutical preparations. Its ultimate fate must depend in great part upon the action of free and combined (proteid) hydrochloric acid upon it. It is, moreover, important to compare the behavior of the ferment in this respect, with the amylolytic ferment of saliva.

An aqueous extract of malt prepared as described, contains but little proteid matter; as a rule 2·0–2·5 c. c. of 0·1 per cent. hydrochloric acid are required to completely saturate the proteid matter contained in 30 c. c. of the neutral malt extract. This point was ascertained by use of the tropaeolin test for free acid as recommended by Danilewsky.* Thus, by way of illustration, in one instance 30 c. c. of carefully neutralized malt extract required the addition of 3·3 c. c. 0·1 per cent. HCl to give the tropaeolin reaction for free acid, and since nothing smaller than 0·003 per cent. free HCl can be detected by this method, it follows, making the proper deduction, that 2·3 c. c. of 0·1 per cent. HCl are required to completely saturate the proteid matter in 30 c. c. of the extract, which would then contain 0·0023 per cent. combined HCl in the form of acid-proteids.*

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* Centralbl. Med. Wiss., 1880; also description in Trans. Conn. Acad., vol. vi, p. 360.

Our first experiments were made to ascertain the influence of small percentages of combined acid on the action of the ferment, viz: the influence of such additions of dilute acid to the neutral malt extract as would completely saturate the proteid matter present, without giving any *free* acid whatever. The results plainly show that the addition of very small quantities of dilute hydrochloric acid to a neutral solution of diastase increases the amylolytic action of the ferment. Following are a few of the results obtained.

A. Malt extract, neutral to test papers, required 2·1 c. c. 0·1 per cent. HCl to completely saturate the proteid matter in 30 c. c.; the fluid was then acid to test papers but contained no free acid.

a. with 30 c. c. of the malt extract

Per cent. combined HCl	Wt. Cu in ¼	Total amount reducing bodies.	Starch converted
0	0·1630 gram	0·3332 gram	29·98 per cent.
0·0024	0·1749	0·3578	32·19

b with 15 c. c. of the malt extract

Per cent. combined HCl	Wt. Cu in ¼	Total amount reducing bodies.	Starch converted
0	0·1285 gram.	0·2516 gram	23·51 per cent.
0·0012	0·1460	0·2976	26·78

B. Malt extract, neutral to test papers, required 3·1 c. c. 0·1 per cent. HCl to saturate the proteid matter in 30 c. c. of the extract.

a. with 30 c. c. of the malt extract

Per cent combined HCl	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted
0	0·1595 gram.	0·3258 gram.	29·32 per cent.
0·0031	0·1761	0·3602	32·11

b. with 15 c. c. of the malt extract

Per cent combined HCl	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted
0	0·1319 gram.	0·2686 gram	24·17 per cent.
0·00155	0·1599	0·3266	29·39

C. With 30 c. c. of malt extract; two distinct preparations.

Per cent. combined HCl.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1575 gram.	0·3214 gram.	28·92 per cent.
0·0023	0·1766	0·3612	32·50
0	0·1681	0·3438	30·94
0·0023	0·1770	0·3620	32·58

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* Doubtless, however, it is not wholly as HCl-proteid, since the extract frequently contains a trace of sodium lactate.

Such a degree of uniformity in the results, makes it evident that the slight accelerating action of the acid-proteid is a constant one under the above conditions, and indeed all of our results in this direction clearly indicate such to be the case.

Although acid-proteid thus accelerates amylolytic action, it is still able under slightly different conditions to retard the action of the ferment and even cause destruction; thus by warming the amount of ferment (30 c. c. malt extract), used in the preceding experiment, at 40° C. for 1 hour with the acid necessary to saturate the proteids, in a total volume of 50 c. c., thus doubling the *percentage* of acid, amylolytic action was found on subsequent neutralization and testing with starch paste, to be very much diminished. The following results obtained with the two malt extracts used in C illustrate this point:

	a.	*b.*	*c.*	*d.*
Per cent HCl	0	0·0046	0	0·0046
Malt extract....	30 c. c.	30 c. c.	30 c. c.	30 c. c.
0·1 per cent. HCl	0	2·3 "	0	2·3 "
H_2O........	20 "	17·7 "	20 "	17·7 "
	50·0 c. c.	50·0 c. c.	50·0 c. c	50·0 c. c.

These solutions were warmed at 40° C. for 1 hour, then neutralizing and equalizing mixtures 'were added, after which amylolytic action was determined by adding starch paste, diluting to 100 c. c., warming at 40° C. for 30 minutes, etc., with the following results, expressed in the percentage of starch converted into sugar.

a.	*b.*	*c.*	*d.*
30·21	21·71	29·25	22·96

Hence, it is apparent that under these conditions, acid-proteids may exert a destructive action on the ferment. This destructive action takes place with considerable degree of rapidity, as is shown by the following experiment, which at the same time illustrates the accelerating action of smaller percentages of combined acid and confirms the preceding experiments.

The neutral malt extract required 2·4 c. c. 0·1 per cent. HCl to combine with the proteid matter in 30 c. c.

	Destructive action.			Accelerating action.	
	1.	*2.*	*3.*	*4.*	*5.*
Malt extract......	30 c. c.	30 c. c.	30 c. c.	30 c. c.	30 c. c.
0·1 per cent. HCl	0	2·4 "	2·4 "	0	2·4 "
H_2O	20 "	17·6 "	17·6 "	70* "	70* "
	50 "	50 "	50 "	100	100
Per cent. HCl ...	0	0·0048	0·0048	0	0·0024

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* Containing one gram of starch.

The amylolytic action of 4 and 5 was tested directly by warming the mixtures at 40° C. for 30 minutes and then determining the amount of reducing bodies. No. 1 (the control) was warmed at 40° C. for 30 minutes, while No. 2 was warmed at the same temperature for 15 minutes and No. 3 for 30 minutes. Neutralizing and equalizing mixtures were added as follows:

	1.	*2.*	*3.*
0·1 per cent. Na_2CO_3...	0	3·5 c. c.	3·5 c. c.
0·1 per cent. HCl......	2·4 c. c.	0	0
0·1 per cent. Na_2CO_3...	3·5 "	0	0

All three were then mixed with one gram of starch and made up to 100 c. c. in order to determine amylolytic action; each solution was neutral and contained the same quantity of sodium chloride as well as the same amount of ferment and starch. Following are the results of all five:

No.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
1	0·1630 gram.	0·3332 gram.	29·98 per cent.
2	0·1253	0·2554	22·98
3	0·1195	0·2434	21·90
4	0·1628	0·3322	29·89
5	0·1749	0·3578	32·19

It is thus seen that with this amount of ferment, 0·0024 per cent. combined acid causes an acceleration in amylolytic action (Nos. 4 and 5) amounting to over 2 per cent. in the quantity of starch converted, while warming the same amount of ferment with the same amount of acid, but under a less degree of dilution, causes a destruction of the ferment amounting to 7 per cent. in the conversion of the starch; 15 minutes longer at 40° C. causes only a slightly increased destruction.

Influence of acid-peptone.

By increasing the amount of proteid matter, larger percentages of hydrochloric acid can be added to a malt extract without retarding the action of the ferment or even interfering with the accelerating action of the smaller percentages. As previously stated, Falk has shown that peptone prevents to a certain extent the retarding action of hydrochloric acid, but it does more than this, it causes acceleration in ferment action not only in neutral solution, as already shown, but in an acid solution likewise, provided there is an excess of peptone present, in which case the acid-peptone compound formed, causes greater acceleration than the same percentage of peptone alone would do if added to a neutral solution of the ferment. The following experiments, which we have repeated many times, testify to the accuracy of this statement:

Per cent. Peptone.	Per cent. combined HCl	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0·2	0	0·2118 gram.	0·4356 gram.	39·20 per cent.
0·2	0·0003	0·2168	0·4460	40·14
0·2	0·0005	0·2115	0·4348	39·13
0·2	0·0030	0·2165	0·4454	40·18
0·2	0·0050	0·2175	0·4478	40·30
0·2	0	0·1730	0·3540	31·86
0·2	0·0003	0·1726	0·3532	31·78
0·2	0·0005	0·1802	0·3688	33·17
0·2	0·0010	0·1794	0·3672	33·04
0·2	0·0030	0·1765	0·3610	32·49
0·2	0·0050	0·1775	0·3632	32·68

Acceleration in amylolytic action is here quite noticeable; the peptone, however, is in considerable excess. As the peptone approaches saturation, retardation commences as is shown by the following experiments; such a point being reached, however, retardation can be completely prevented by increasing the amount of peptone.

Per cent. Peptone	Per cent. combined HCl.	Wt. Cu in ¼	Total amount reducing bodies.	Starch converted.
0·2	0	0·1716 gram.	0·3508 gram.	31·57 per cent.
0·2	0·008	0·1732	0·3424	30·81
0·2	0·010	0·1631	0·3334	30·00
0·2	0·012	0·1442	0·2940	26·46
0·5	0	0·1633	0·3338	30·04
0·5	0·012	0·1652	0·3376	30·38
0·5	0·025	0·1170	0·2384	21·45

The peptone employed in this last experiment required 11·8 c. c. of 0·1 per cent. hydrochloric acid to completely saturate 0·2 gram, consequently in the fourth experiment the peptone was more than completely saturated with acid, but the amount of free acid could have been only 0·0002 per cent. In the last experiment, however, retarding action is due wholly to acid-peptone, no free acid at all being present. Increasing the percentage of combined acid still further, the proteid matter at the same time being just saturated, causes far greater retardation in the action of the ferment.

Per cent. Peptone.	Per cent. combined HCl.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0·5	0	0·1607 gram.	0·3282 gram.	29·53 per cent.
0·5	0·012	0·1661	0·3394	30·54
0·5	0·015	0·1670	0·3412	28·70
0·5	0·025	0·1202	0·2448	22·03
0·5	0·035*	0·0317	0·0674	6·06

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* With this percentage the peptone is exactly saturated with acid.

All of these results show acceleration under the influence of small percentages of combined acid, followed, as the percentages are increased, by decided retardation, thus agreeing with the results previously obtained with the salivary ferment.

The destructive action of small percentages of acid-peptone on the ferment is not great in the presence of an excess of peptone, but as the peptone approaches saturation its destructive power is increased, and when completely saturated with acid, a moderate amount of peptone so combined will quickly and completely destroy the ferment. This is plainly shown in the following series of experiments:

	1.	*2.*	*3.*	*4.*
Malt extract.... ...	15 c. c.	15 c. c.	15 c. c.	15 c. c.
Peptone sol.*........	20	20	20	20
HCl 0·1 per cent.....	0	1	2	4
H_2O............ ...	15	14	13	11
	50	50	50	50
Peptone	0·1 per cent.	0·1 per cent.	0·1 per cent.	0·1 per cent.
Combined HCl.... .	0	0·002	0·004	0·008

These mixtures were warmed at 40° C. for 30 minutes. 20 c. c. of the peptone solution together with 15 c. c. of the malt extract required 4·0 c. c. of 0·1 per cent. hydrochloric acid to saturate the proteid matter, consequently in the above mixtures, 2 and 3 contained a large excess of uncombined peptone, while in 4 the proteid matter was just saturated.

Neutralizing and equalizing mixtures were added to each as follows:

	1.	*2.*	*3.*	*4.*
0·1 per cent. Na_2CO_3 ¦ ...	0	1·5 c. c.	3 c. c.	5·9 c.c.
{ 0·1 " Na_2CO_3	5·9 c. c.	4·4	2·9	0
{ 0·1 " HCl..........	4·0	3·0	3·0	0

The solutions were now, on being diluted to 100 c. c., in every respect equal. Their amylolytic power on being tested with starch paste was as follows:

No	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
1	0·1595 gram	0·3258 gram.	29·32 per cent.
2	0 1527	0·3118	28·06
3	0·1239	0·2522	22·60
4	trace.		

Hence, when the proteid matter present is only one-quarter saturated with acid, the acid-peptone so formed may exert some destructive action on the ferment; when half saturated, destructive action is more pronounced; when wholly saturated it is, under the above conditions, complete.

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* The peptone solution contained 0·250 gram peptone in 100 c. c. water and was made neutral to test papers; 20 c. c. therefore contained 0·050 gram peptone.

Frequently, such small percentages of combined acid as the above will have no retarding effect whatever on amylolytic action, though if the ferment be warmed for half an hour with the same percentage of peptone and combined acid, its subsequent amylolytic power will be much reduced, owing to a partial destruction of the ferment. This is well illustrated by the following series of experiments:

	A.			B.		
	1.	*2.*	*3.*	*4.*	*5.*	*6.*
Malt extract	15 c. c	15 c. c.	15 c. c.	15 c. c	15 c c.	15 c. c.
0·1 per cent HCl	0	1	2	0	2	4
Peptone sol. 0·5%	10	10	10	20	20	20
H_2O	25	24	23	65*	63*	61*
	50	50	50	100	100	100
Combined HCl	0	0·002%	0·004%	0	0·002%	0·004%
Peptone	0·1%	0·1	0·1	0·1%	0·1	0·1

Nos. 4, 5 and 6 were warmed directly, with the starch, for 30 minutes at 40° C. and the reducing bodies determined. Nos. 1, 2 and 3 were warmed at 40° C. also for 30 minutes, then neutralizing and equalizing mixtures were added, the solutions diluted to 100 c. c. and warmed with 1 gram of starch for 30 minutes at 40° C. The following results show the amylolytic power of the six solutions:

	No	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
A.	1	0·1482 gram.	0·3024 gram.	27·21 per cent.
	2	0·1412	0·2876	25·88
	3	0·1351	0·2754	24·78
B.	4	0·1594	0·3256	29·30
	5	0·1628	0·3321	29·91
	6	0·1605	0·3282	29·52

In series A, we see a gradual decrease in the amylolytic power of the solutions; this can be due to nothing but the destructive action of the acid-peptone compound, for the solutions are in every respect alike, being in the same degree of dilution and containing the same amount of sodium chloride, etc. The destruction is not great, as the peptones are in considerable excess. In series B, where the ferment is exposed to the direct action, in the presence of the starch, of the same percentage of peptone and acid there is no retardation of amylolytic action whatever; presumably because of the rapid action of the ferment and the slow retarding action of the acid-peptone.

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* Containing 1 gram of starch.

Influence of free acid on the amylolytic action of diastase.

As might be expected, free hydrochloric acid, even in very small quantity at once stops the amylolytic action of this ferment, quickly destroying it. It is interesting, however, to compare the action of very small percentages of free acid with results obtained in like manner with the salivary ferment. The first experiment gave the following results: the malt extract used, required per 30 c. c., 2·4 c. c. 0·1 per cent. hydrochloric acid to saturate the proteid matter.

Per cent combined HCl.	Per cent. free HCl.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0	0·1378 gram.	0·2808 gram.	25·27 per cent.
0·0024	0	0·1584	0·3236	29·12
0·0024	0·001	0·1486	0·3032	27 29
0·0024	0·003	0·0805	0·1642	14·77

With another malt extract not so active, but containing the same percentage of acid-proteids, the following results were obtained:

Free HCl.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·0780 gram.	0·1592 gram.	14·32 per cent.
0·0003 per cent.	0·0719	0·1470	13·23
0·0005	0·0631	0·1294	11·64
0·0020	0·0380	0·0796	7·16
0·0030	0·0091	0·0222	1·99

From these two series of results it is quite evident that a very small percentage of free hydrochloric acid will stop the amylolytic action of this ferment. The main action of the acid is that of destruction, killing the ferment very quickly. The following is a sample of several experiments tried, to ascertain how far retardation is due to destruction of the ferment.

	1.	*2.*	*3.*	*4.*
Neutral malt extract	30 c. c.	30 c. c.	30 c. c.	30 c. c.
0·1 per cent. HCl to saturate proteids	2·2 "	2 2 "	2·2 "	2·2 "
0·1 per cent. HCl for free acid	0	0·5 "	1·0 "	1·5 "
H_2O	17·8 "	17·3 "	16·8 "	16 3 "
	50 "	50 "	50·0 "	50·0 "
Per cent. free HCl	0	0·001	0·002	0·003

These solutions were warmed at 40° C. for 30 minutes, then neutralizing and equalizing mixtures were added and the amylolytic power determined. No. 1 converted the usual amount of starch into sugar, but No. 2 showed only a trace of amylolytic power and Nos. 3 and 4 none at all. Evidently then 0·001 per cent. of free acid had all but completely destroyed the ferment by 30 minutes warming at 40° C.

In conclusion then, we have to notice a greater susceptibility on the part of this ferment to the action of acid-proteids and free acid than the salivary ferment. Whether this latter point constitutes any real difference, it is hard to say, since the apparent increase in amylolytic action noted in the presence of traces of free acid in the case of saliva (0·0001–0·0006 per cent. free HCl) involve such small quantities as to make the results somewhat questionable,* since such very small additions of acid might perhaps be used up by the phosphates or other salts present. But taking the evidence of the results and comparing them with results obtained in like manner with the diastase of malt, it would certainly appear that the latter is more susceptible to the action of free acid than the salivary ferment, though both are very readily destroyed by a few thousandths of one per cent. of free HCl.

In other respects, the ferment of malt behaves similarly to the ferment of saliva; both act better in a neutral than in an alkaline solution; proteid matter too, prevents the retarding action of alkaline carbonate and thus, as in the case of saliva, the action of a given percentage of sodium carbonate on diastase is dependent in part, upon the concentration of the fluid and the consequent amount of proteid matter present. Neutral peptone, moreover, exerts a direct stimulating effect on the amylolytic action of neutral diastase. Greatest amylolytic action, as in the case of saliva, is, however, observed in the presence of proteid matter partially saturated with acid, but larger percentages of acid-proteids may cause complete destruction of the ferment. The accelerating action of proteid matter is in great part due to its power of combining with both acid and alkaline carbonate, but in addition we cannot but recognize a direct stimulation of the ferment, as in the action of neutral peptone on a neutral solution of diastase.

Lastly, it is evident from these results, that diastase taken into the stomach must sooner or later be completely destroyed, by either the free acid or the large percentage of acid-proteids; but in the first stage of digestion, in the absence of free acid and under the protecting influence of proteid matter the conversion of starch into sugar may still go on, though soon destined to feel the effects of the gradually increasing percentage of combined acid.

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* See Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 370.

V.—Influence of Certain Therapeutic and Toxic Agents on the Amylolytic Action of Saliva. By R. H. Chittenden and H. M. Painter, B.A., Ph.B.

Few attempts have been made to ascertain, experimentally, the influence of therapeutic and toxic substances on amylolytic action. Yet in view of the important part which the ferment of saliva plays in the digestive processes of the body and in view likewise of the great susceptibility of the ferment, it would seem especially desirable to obtain accurate data regarding the effects of many substances on its amylolytic power.

While many laborious investigations have, from time to time, been undertaken to ascertain the influence of some one or more substances on the metabolism of the body, the influence of the same substances on the digestive processes has apparently been very little considered, with the exception, however, of the more common alkali and alkali-earth salts. Likewise too, the possible action of many toxic substances on the digestive processes, as in chronic cases of poisoning, has with a few exceptions been almost entirely ignored; yet in both of these instances it is possible that much light might be obtained by a knowledge of the influence of individual substances upon proteolytic and amylolytic action.[*]

With these thoughts in mind, the present investigation was undertaken, and the results which we present here plainly show the importance of the work.

In selecting substances for study, we have chosen not only those noted for therapeutic or toxic power, but also those possessed of antiseptic or germicidal properties; our object being to see how far the unformed ferment of the saliva corresponds, in its behavior towards these bodies, with the formed or organized ferments.[†] Moreover, only neutral bodies could be experimented with, since the smallest quantity of either free acid or alkali would exert its own peculiar destructive action on the ferment.* In view of this fact also, we have invariably used chemically pure salts and those frequently recrystallized to be sure of the absence of deleterious impurities.

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[*] An interesting table of comparisons by Wernitz shows the relative action of several therapeutic agents, on the various enzymes of physiological interest.—Brunton's Pharmacology, p. 86.

[†] A difference in action by the same substance upon formed and unformed ferments is, as stated by Brunton, a fact of great importance, for upon it may depend a useful application of the substance in medicine; thus creosote, which has but a slight action upon pepsine and ptyaline, will kill bacteria in a dilution of 1 to 1000, and thus this agent can be used to arrest fermentation in the stomach depending on the presence of low organisms, while the proteolytic action of the digestive ferment is but little interfered with.—Brunton, p. 87.

Method employed.

A few preliminary experiments clearly indicated that the presence of very small percentages of foreign substances exercise a decided effect on the amylolytic action of saliva, and thus the investigation resolved itself into a study, not of the percentages requisite to *completely* hinder the power of the ferment, under given conditions, but of the relative action of small percentages on the amylolytic power of the ferment. This seemed to us the more important, since we soon found that substances which, present in comparatively large amount tended to hinder amylolytic action, would when present in small quantities actually increase the activity of the ferment. Hence, we deemed it best to use accurate quantitative methods for determination of amylolytic action; such as would indicate small variations with certainty.

The experiments were made in series, in which one digestion of each series served as a control for comparison. The volume of each digestive mixture was 100 c. c., in which was present 1 gram of perfectly neutral potato starch, previously boiled with a portion of the water, 10 c. c. of a diluted neutral saliva† and a given quantity of the substance to be experimented with. The mixtures were warmed at 40° C. for 30 minutes, after which further action of the ferment was stopped by heating the solution to boiling. The extent of amylolytic action was then ascertained by determining in one-fourth of the solution, the amount of reducing substances by Allihn's‡ gravimetric method. From the amount of reduced copper thus obtained, the total amount of reducing bodies was calculated (as dextrose), from which in turn was calculated the percentage of starch converted.

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* Chittenden and Smith, Transactions Conn. Acad. Arts and Sciences, vol. vi, p. 343.

† The saliva was human, mixed saliva, freshly collected. It was prepared for use by being filtered, made exactly neutral, then diluted in the proportion of 1 : 5. Thus in each digestion there were present 2 c. c. of undiluted saliva.

‡ Zeitschrift für Analytische Chemie, Jahrgang xxii, p. 448.

Mercuric chloride.

Sternberg* places mercuric chloride first in the list of germicides; its presence to the extent of 0·003 per cent. being sufficient to prevent the development of the micrococcus of pus, while 0·005 per cent. destroys the vitality of the same bacterial organism.

To our surprise the salt acts even more energetically on the unorganized ferment of the saliva, as the following results show:

$HgCl_2$	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·2385 gram.	0·4920 gram.	44·28 per cent.
0·0005 per cent.	0·1277	0·2500	22·50
0·0010	0·0925	0·1880	16·92
0·0020	0·0395	0·0824	7·41
0·0030	0·0060		
0·0040	0		

It is evident that the ferment of saliva is very susceptible to the action of this poison, and we have repeated the experiment, using still smaller percentages, with the following results:

$HgCl_2$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1635 gram.	0·3340 gram.	30·06 per cent.
0·0001 per cent.	0·1610	0·3288	29·59
0·0002	0·1570	0·3204	28·83
0·0003	0·1545	0·3152	28·36

The smallest possible addition, therefore, of mercuric chloride diminishes the amylolytic power of saliva, in proportion to the amount of mercury salt added.

Mercuric bromide, mercuric iodide and mercuric cyanide.

These salts of mercury, vigorous in their action as poisons, and the two former as germicides likewise, would be expected from analogy to act similarly to the chloride. Such we find to be the case with the bromide and iodide, but with the cyanide there is to be noticed, to a slight extent, an action which we find common to many substances, viz: increasing the amylolytic power of the ferment

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* Amer. Jour. Med. Sciences, April, 1883, p. 321.

For the action of the various salts studied in this work, on the organized ferments, see also Marcus and Pinet in Compt. Rend. Soc. de Biolog., 1882, pp. 718–724, or abstract in Jahresbericht für Thierchemie, 1882, p. 515; also Ch. Richet in Compt. Rend., vol. xcvii, pp. 1004–1006, or in Jahresbericht für Thierchemie, 1883, p. 418, and Robert Koch, Jahresbericht für Thierchemie, 1881, p. 471, N. Jalan de la Croix, Jahresbericht für Thierchemie, 1881, p. 476. Brunton's Pharmacology, p. 96, when present in one percentage and diminishing it when the percentage is increased. On account of the insolubility of mercuric iodide and bromide, these salts were dissolved in water containing potassium iodide and sodium chloride respectively, in such proportion that the various digestive mixtures contained the same percentages of these salts as they did of the mercury salts.* Following are the results obtained:

Mercury salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1295 gram.	0·2636 gram.	23·72 per cent.
$HgBr_2$			
0·0005 per cent.	0·1150	0·2344	21·09
0·0010	0·0770	0·1572	14·14
0·0020	0·0340	0·0720	6·48
HgI_2			
0·0010	0·1257	0·2560	23·04
0·0020	0·1180	0·2404	21·63
$Hg(CN)_2$			
0·0005	0·1375	0·2800	25·20
0·0010	0·1445	0·2944	26·49
0·0020	0·1242	0·2528	22·75
0·0030	0·1252	0·2552	22·96
0	0·1319	0·2684	24·15
$Hg(CN)_2$			
0·0500	0·1025	0·2084	18·75

In this series of results, it is to be noticed that mercuric bromide is the most energetic in its hindering action; 0·0005 per cent. being even more effective than 0·002 per cent. of the iodide. With the cyanide, however, the first two percentages stimulate or in some way give rise to an increased amylolytic action and even 0·050 per cent. of the salt does not retard the action of the ferment as much as 0·001 per cent. of mercuric bromide.

Cupric sulphate.

With this salt the following results were obtained:

$CuSO_4+5H_2O$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1712 gram.	0·3500 gram.	31·50 per cent.
0·0005 per cent.	0·1445	0·2944	26·49
0·0020	0·0530	0·1096	9·86
0·0100	0·0250	0·0540	4·86
0·0250	0		

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* Apparently, the double salts so formed act as vigorously as the mercury salt alone could do.

The hindering action of the copper salt is nearly as pronounced as that of mercuric chloride and even more so than the bromide and iodide of mercury.

Lead acetate.

With this salt, the smaller percentages experimented with show a slight stimulating action; but the larger percentages fail to retard the amylolytic action of the ferment as the preceding salts.

$Pb(C_2H_3O_2)_2+3H_2O$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1630 gram.	0 3332 gram.	29·98 per cent.
0·0003 per cent.	0·1642	0·3356	30·20
0·0005	0·1635	0·3340	30·06
0·0010	0·1635	0·3340	30·06
0·0020	0·1595	0·3256	29·30
0·0050	0·1395	0·2840	25·56
0·0100	0·1402	0·2856	25·70

A second series of experiments, with still larger percentages of the lead salt, gave the following results:

$Pb(C_2H_3O_2)_2+3H_2O$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1742 gram.	0·3561 gram.	32·07 per ce
0·05 per cent.	0·1735	0·3548	31·93
0·10	0·1720	0·3516	31·61
0·30	0·1657	0·3384	30·45
0·50	0·1555	0·3172	28 54
1·00	0·1375	0·2800	25·20
3·00	0·0785	0·1600	14·40
5·00	0·0490	0·1016	9·14

Thus, the presence of even five per cent. of lead acetate fails to completely prevent amylolytic action.

Arsenious oxide.

Owing to the comparative insolubility of this substance in neutral fluids, small percentages only could be experimented with. With these, the following results were obtained:

As_2O_3.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1475 gram.	0·3004 gram.	27·03 p
0·0003 per cent.	0·1507	0·3072	27·64
0·0005	0·1537	0·3136	28·22
0·0010	0·1475	0·3004	27·03
0·0020	0·1570	0·3204	28·83
0·0050	0·1390	0·2832	25·48
0·0900	0·1605	0·3276	29·48

Although the results obtained do not wholly accord with each other they still plainly show that arsenious acid, to the extent present in these experiments, stimulates the amylolytic action of the ferment; a fact which might be expected, assuming that the acid combines with the proteids of the saliva, for as has been elsewhere[*] shown, acid-proteids when present in not too large an amount increase the amylolytic action of the salivary ferment.

Schäfer and Böhm[†] state that arsenious acid has no influence whatever on the conversion of starch into sugar by a glycerine extract of the pancreas. Possibly they sought only for retarding action, or it may be that the pancreatic ferment differs in this respect from the ferment of saliva.

Arsenic acid.

This substance being still more acid than the preceding, might naturally be expected to diminish amylolytic action, when present in quantities which in the preceding would increase the activity of the ferment; and indeed there is to be seen in the results, a slight increase, followed by a rapid decrease of amylolytic action.

H_3AsO_4.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted
0	0·1755 gram.	0·3588 gram.	32·29 per cent.
0·0005 per cent.	0·1765	0 3608	32·47
0·0010	0·1635	0·3340	30·06
0·0030	0·0310	0·0660	5·94
0·0050	0		

With 0·005 per cent. of arsenic acid present in the fluid, no reducing bodies were formed in the thirty minutes of the experiment, but the solution did become clear, showing the formation of soluble products. The same fact was observed in the presence of larger percentages of the acid; the starch solution becoming clear, after the addition of saliva, even in the presence of one per cent. of the acid, although, as before, no reducing bodies were formed.

Ammonium arsenate.

With this salt the following results were obtained:

$(NH_4)_3AsO_4$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1527 gram.	0·3112 gram.	28·08 per cent.
0·0005 per cent	0·1620	0·3308	29·77
0·0010	0·1630	0·3340	30·06
0·0050	0·1675	0·3420	30·78
0·0150	0·1745	0·3568	32·11
0·0250	0·1700	0·3476	31·28

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[*] Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 343.

[†] Abstract in Jahresbericht für Thierchemie, 1872, p. 365.

In a second series, larger percentages were used with the following results:

$(NH_4)_3AsO_4$.	Wt Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1712 gram.	0·3500 gram.	31·50 per cent.
0·05 per cent.	0·1475	0·3004	27·03
0·10	0·1147	0·2332	20·98
0·50	0·0165	0·0368	3·31
1·00	The solution became clear but no reducing bodies were formed.		

With this salt, a very decided stimulation of the ferment is to be observed in the presence of small percentages, while increased amounts of the salt ultimately stop diastatic action.

Potassium antimony tartrate.

Two series of experiments were tried with this salt, with the following results:

$K(SbO)C_4H_4O_6$.	Wt. Cu in ¼	Total amount reducing bodies.	Starch converted.
0	0·1540 gram.	0·3144 gram.	28·29 per cent.
0·001 per cent.	0·1610	0·3288	29·59
0·005	0·1660	0·3392	30·52
0·010	0·1760	0·3600	32·40
0·050	0·1760	0·3600	32·40
0·100	0·1745	0·3568	31·11
0·200	0·1750	0·3580	32·22
0	0·1545	0·3152	28·36
0·10	0·1850	0·3788	34·09
0 30	0·1640	0·3352	30·16
0·50	0·2565	0·5304	47·73
1·00	0·1570	0·3204	28·83
2·00	0·1232	0·2504	22·53
5·00	0·0470	0·0976	8·78

Here we have an illustration, more forcible than with any other salt, of the power possessed by many substances of both increasing and diminishing the action of the ferment. One* of us has for some time held that the addition of very small quantities of hydrochloric acid to neutral saliva tends to increase the amylolytic power of the ferment; that this takes place even when the proteids present are completely saturated with the acid, or in other words when there is present a *very small* amount of *free* acid, provided the acid-proteids are not present in too large an amount. It is well known that free hydrochloric acid, when present to the extent of a few thousandths of one per cent. completely stops the action of the ferment. Langley and Eves* make this divergence of action of one and the same substance a ground for questioning the accuracy of such a view, for, say they, "since 0·0015 per cent. HCl decreases amylolytic action it seems very unlikely that 0·0005 per cent. should increase it." The action of many neutral salts here experimented with, where both stimulation and retardation are obtained, plainly show that such a double action, dependent simply on quantity is not an impossible one.

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* Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 360.

Stannous chloride.

With this salt very marked results were obtained as follows:

$SnCl_2$	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1475 gram.	0·3004 gram.	27·03 per cent.
0·0003 per cent.	0·1582	0·3232	29·08
0·0010	The solution became clear, but no reduction.		
0·0050	The starch was not at all altered in appearance.		

Here there is stimulation, followed by rapid and complete stopping of amylolytic action.

Zinc sulphate.

$ZnSO_4+7H_2O$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1495 gram.	0·3048 gram.	27·43 per cent.
0·0003 per cent.	0·1490	0·3040	27·36
0·0005	0·1510	0·3088	27·79
0·0010	0·1475	0·3004	27·03
0·0020	0·1440	0·2936	26·42
0·0050	0·1360	0·2772	24·94
0·0100	0·1260	0·2576	23·18
0	0·1375	0·2800	25·20
0·05 per cent.	0·0775	0·1480	13·32
0·10	0·0650	0·1332	11·98
0·30	0·0450	0·0936	8·42
0·44	0		

These two series of experiments plainly show a gradually diminished amylolytic action, as the percentage of the zinc salt is increased, until with 0·4 per cent. a complete stoppage is effected.

Kjeldahl† found a like retarding action on the addition of zinc sulphate to a malt extract.

In this connection it is interesting to note that Sternberg‡ finds zinc sulphate devoid of germicide value, even when used in the proportion of 20 per cent.

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* Journal of Physiology, vol. iv, No. I.

† Jahresbericht für Thierchemie, 1879, p. 382.

‡ Amer. Jour. Med. Sciences, April, 1883, p. 330.

Ferric chloride.

With this salt we obtained the following results:

Fe_2Cl_6.	Wt. Cu in ¼	Total amount reducing bodies.	Starch converted
0	0·1740 gram.	0·3560 gram.	32·01 per cent.
0·0005 per cent.	0·1597	0·3260	29·31
0·0020	0·0437	0·0908	8·17
0·0100	0·0095	0·0236	2·12
0·0250	0		

Sternberg states that tincture of ferric chloride is effective as a germicide (upon micrococcus) when present to the extent of 4 per cent. On the unformed ferment of the saliva, it is, as the results show, much more active, its hindering action being directly proportional to the percentage of iron salt present.

Ferrous sulphate.

With this salt of iron quite different results were obtained; and as we wished simply to compare its action with that of the ferric salt, only very small percentages were experimented with.

$FeSO_4+7H_2O$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1245 gram.	0·2532 gram.	22·78 per cent.
0·0005 per cent.	0·1037	0·2108	18·97
0·0020	0·1323	0·2692	24·22
0·0100	0·1365	0·2780	25·02

Here there is decided stimulation with the two larger percentages, while the smallest per cent. shows an apparent decrease of amylolytic action.

Kjeldahl* found that this salt exercised a strong hindering action on the amylolytic ferment of malt.

Potassium permanganate.

Sternberg places this salt next to mercuric chloride in germicide value, it being efficacious in 0·12 per cent. With the unformed ferment of the saliva it is likewise active, although no more so than many other salts experimented with. Following are the results:

$K_2Mn_2O_8$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1475 gram.	0·3004 gram.	27·03 per cent.
0·005 per cent.	0·1012	0·2060	18·54
0·025	0		

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* Jahresbericht für Thierchemie, 1879, p. 382.

Magnesium sulphate.

With this salt we obtained the following results:

$MgSO_4+7H_2O$	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1475 gram.	0·3004 gram.	27·03 per cent.
0·025 per cent.	0·1597	0·3260	29·34
0·500	0·0510	0·1056	9·50

Here, there is a slight increase of diastatic action with the smallest percentage, while 0·5 per cent. of the salt greatly retards the action of the ferment.

Pfeiffer* has likewise noticed the retarding effect of this salt on salivary digestion.

Potassium cyanide.

This salt, so powerful as a poison, was found to have a decided effect also on the salivary ferment, causing a rapid decrease in amylolytic action.

KCN	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1245 gram.	0·2532 gram.	22·78 per cent.
0·0005 per cent.	0·1080	0·2200	19·80
0·0010	0·0896	0·1828	16·45
0·0030	0·0330	0·0700	6·30

With 1·0 per cent. and even with 5·0 per cent. of potassium cyanide, the starch solutions became clear on the addition of saliva, showing that the ferment was able to effect some change, although in neither case were any reducing bodies formed.

It is our intention at some future time, to study the exact nature of the products formed under such conditions. The ferment appears to be peculiarly affected; for while a very small percentage of a substance like potassium cyanide or borax will completely prevent the formation of reducing bodies, increasing the amount of substance added a hundred-fold, has no effect on the clearing up of the starch solution by the ferment. Some light may be thrown upon the nature of the ferment or its mode of action.

Potassium ferrocyanide.

A preliminary experiment showed that this salt was less active than the cyanide and therefore larger percentages were used, with the following results:

$K_4Fe(CN)_6+3H_2O$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1417 gram.	0·2884 gram.	25·96 per cent.
0·025 per cent.	0·1497	0·3052	27·46
0·100	0·1375	0·2800	25·20
0·250	0·1025	0·2084	18·80

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* Centralbl. Med. Wiss., 1885, p. 328, abstract.

Here, unlike the cyanide, there is stimulation of the ferment. Retardation of amylolytic action requires much larger percentages; thus 1·0 per cent. of ferrocyanide completely prevented the formation of reducing bodies, although soluble starch was apparently formed, as also in the presence of 5·0 per cent. of the salt.

Potassium ferricyanide.

The action of this salt is almost identical with that of the ferrocyanide.

$K_6Fe_2(CN)_{12}$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0 1417 gram.	0·2884 gram.	25·96 per cent.
0·025 per cent.	0·1515	0·3088	27·79
0·100	0·1295	0·2636	23·72
0 250	0·0975	0·1984	17·85

Like the ferrocyanide, this salt in 1·0 and 5·0 per cent. solutions allows the partial conversion of starch into soluble products, but no reducing bodies are formed.

Potassium nitrate and potassium chlorate.

Potassium salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1513 gram.	0·3080 gram.	27·72 per cent.
KNO_3.			
0·20 per cent.	0·1550	0·3164	28·47
0·50	0·1528	0·3108	27·97
1·00	0·1462	0·2976	26·78
$KClO_3$.			
0·20	0·1581	0·3228	29·05
0·50	0·1580	0·3228	29·05
1·00	0·1600	0·3268	29·41

With 5·0 per cent. of the salts, the following results were obtained:

Potassium salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1672 gram.	0·3416 gram.	30·74 per cent.
KNO_3 (5·0 pr. ct.)	0·1559	0·3176	28·58
$KClO_3$ (5·0 pr. ct.)	0·1351	0·2752	24·76

With these two salts it is very obvious that small fractions of one per cent. decidedly increase amylolytic action and that potassium chlorate is the more energetic of the two in this respect. With one per cent. of the salts, potassium chlorate still shows increased action, while the nitrate causes a decrease of amylolytic activity; in the presence of 5 per cent. of the salts, on the other hand, potassium chlorate causes the greatest decrease in ferment action.

Of these two oxidizing agents, potassium chlorate does not appear to have been hitherto experimented with, but with potassium nitrate

O. Nasse* found increased amylolytic action with human saliva in the presence of 4·0 per cent. of the salt. Possibly this difference in our results is dependent in part upon difference in the relative amount of salt and ferment.

Sodium tetraborate $[Na_2B_4O_7+10H_2O]$.

With this salt experiments were tried with quantities varying from 0·050 to 3·0 per cent. and in each instance the starch was dissolved, but no reducing bodies whatever were formed. Dumas† has previously noted a like retarding effect on the diastatic action of emulsin, diastase and other like ferments. Sternberg states that this salt is without germicide value, even though used in a saturated solution; its antiseptic power, i. e. its capacity for preventing the multiplication of bacterial organisms, is, however, considerable.

Potassium bromide and potassium iodide.

These two common therapeutic agents gave the following results:

Salt used.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1483 gram.	0·3020 gram.	27·18 per cent.
KBr.			
0·5 per cent.	0·1566	0·3192	28·72
3·0	0·1450	0·2956	26·60
5·0	0·1314	0·2668	23·51
KI.			
0·5	0·1550	0·3164	28·47
3·0	0·1557	0·3172	28·54
5·0	0·1467	0·2984	26·85

Both of these salts show a stimulating action which is more persistent in the case of the iodide than with the bromide; 5·0 per cent. of the bromide causes a marked diminution of amylolytic action.

Sodium chloride.

Previous experiments have been tried with this salt by several investigators, notably by O. Nasse‡ and E. Pfeiffer.§ The former found that the presence of 4·0 per cent. of the salt [the only percentage experimented with] caused an increase in the ferment action of saliva [128 : 100]; the latter experimenter likewise found that the presence of the salt, in concentrations up to 2 per cent., greatly increased the amylolytic action of saliva.

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* Pflüger's Archiv. für Physiologie, vol. xi, p. 150.

† Berichte der deutsch. Chem. Gesell., vol. v, p. 826.

‡ Pfluger's Archiv für Physiologie, vol. xi, p. 155.

§ Centralbl. med. Wiss., 1885, p. 329.

Our results with different percentages are as follows:

NaCl	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1660 gram.	0·3392 gram.	30·52 per cent.
0·3 per cent.	0·1765	0·3608	32·17
0 5	0·1750	0·3580	32 22
1 0	0·1715	0·3501	31·53
2·0	0·1715	0·3504	31·53
3·0	0·1770	0·3620	32·58
5·0	0·1630	0·3332	29·98

These accord with the results mentioned above and show, moreover, that with 5·0 per cent. of the salt, hindering action just commences. Increasing the amount of salt beyond this point, however, only slowly diminishes the action of the ferment; thus, in the presence of 10·0 per cent. of the salt, 22·78 per cent. of starch was converted into sugar, while without it 25·20 per cent. of starch was converted.

Morphine sulphate.

With this alkaloid O. Nasse* has experimented, using, however, the acetate. He found that the presence of 0·1 per cent. of the salt caused a slight increase in the diastatic action of saliva (109 : 100). Our results with the sulphate of morphine are as follows:

Alkaloid salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1245 gram.	0·2532 gram.	22·78 per cent.
0·05 per cent.	0·1415	0·2880	25·92
0·50	0·1605	0·3276	29·48
2·00	0·1428	0·2908	26·17

The stimulating action of the alkaloid salt up to 2·0 per cent. is very apparent.

Quinine sulphate.

With the acetate of this alkaloid, Nasse found, by the use of 0·1 per cent., an increase in the starch-converting power of the saliva (115 : 100). With the sulphate we obtained the following results:

Alkaloid salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1358 gram.	0·2768 gram.	24·91 per cent.
0·05 per cent.	0·1475	0·3004	27·03
0·50	0·1355	0·2760	24·84
2·00	0·0981	0·1996	17·96

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* Pflüger's Archiv, vol. xi, p. 161.

In accord with Nasse's result we see that 0·05 per cent. increases the amylolytic action of the ferment. This we verified by an additional experiment which led to a like result, although not showing so great a difference as the preceding one; thus, while the saliva alone converted 23·72 per cent. starch into reducing bodies, the presence of 0·05 per cent. of quinine sulphate led to the conversion of 24·58 per cent. of starch. Voit, as quoted by v. Boeck,* has stated that quinine is without influence on the ferment of saliva.

Cinchonine sulphate.

With this alkaloid, previous experiments have not to our knowledge been tried. Our results are as follows:

Alkaloid salt.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1358 gram.	0·2768 gram.	24·91 per cent.
0·05 per cent.	0·1452	0·2960	26·64
0·50	0·1455	0·2964	26·67
2·00	0·1440	0·2936	26·42

Cinchonidine sulphate.

This alkaloid, like the cinchonine, shows a steady accelerating action on the ferment.

Alkaloid salt.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1358 gram.	0·2768 gram.	24·91 per cent.
0·05 per cent.	0·1505	0·3068	27·61
0·50	0·1460	0·2976	26·78
1·75	0·1498	0·3056	27·50

The cinchona group of alkaloids thus show throughout an accelerating influence on amylolytic action, most pronounced in the case of cinchonidine. These alkaloids have long been known to prevent putrefaction and to check alcoholic fermentation and Binz† has demonstrated that this antiseptic action, in the case of quinine at least, is due to the poisonous influence exerted by the latter upon the fungi which are the immediate cause of the putrefactive changes. Couzen, moreover, has shown that the action of cinchonine on infusoria and on fermentation is similar to that of quinine, but weaker. Hence there is no similarity of action whatever, on the two kinds of ferments.

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* Zeitschrift für Biologie, vol. vii, p. 428.

† Virchow's Archiv, vol. xlvi, 1869, p. 68.

Atropine sulphate.

With this alkaloid we obtained the following results:

Alkaloid salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted
0	0·1485 gram.	0·3028 gram.	27·25 per cent.
0·025 per cent.	0·1460	0·2976	26·78
0·050	0·1410	0·2872	25·62
0·200	0·1530	0·3124	28·11
0·500	0·1407	0·2864	25·77
1·000	0·1475	0·3004	27·03
2·000	0·1245	0·2532	22·78

The main action of the smaller percentages of this alkaloid seems to be a slightly hindering one, although there are one or two irregularities in the results which are not readily explainable. In the presence of 2·0 per cent. of atropine sulphate there is a decided diminution in amylolytic action.

In connection with this alkaloid we have to note some recent experiments of Stolnikow* of St. Petersburg. This investigator, producing artificial fever in dogs by the injection of putrid matter into the blood found, first, that the salivary and pancreatic secretions were for a time increased in amount and then rapidly diminished and finally entirely ceased. This latter action of the septic poison, Stolnikow found to be very persistent and he moreover states that in physiological action the septic poison resembles atropine. Furthermore that artificial fever, produced as described, exercises a decided influence on the *content of ferments* in the pancreatic gland; that in fevers of short duration (2–10 hours) the extract of this gland has a more energetic ferment action than the normal extract, while in fevers of long duration the corresponding extract has a much weaker action. Overlooking now the physiological explanation suggested for these facts we come to the chemical one, viz: that the septic poison possibly exerts either a destructive or hindering influence on the ferment or its action. In support of this view, Stolnikow found that large quantities of the poison did weaken the amylolytic and proteolytic action of extracts from the pancreatic gland, although small quantities of the septic ferment were without action. Likewise, Stolnikow states that small quantities of atropine sulphate are without action on a glycerine extract of the pancreas, but by adding to 10 c. c. of a glycerine extract, 5 c. c. of a 3·0 per cent. atropine sulphate solution and allowing the mixture to stand at the ordinary temperature for 10 hours, then on testing the amylolytic power of the ferment its action was found to be much weaker than the control. From this fact, Stolnikow considers that the septic poison acts upon the ferment outside the body in a manner similar to atropine.

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* Beiträge zur Lehre von der Function des Pancreas im Fieber. Virchow's Archiv, vol. xc, p. 389, 1882.

Now it is obvious, in view of the extreme susceptibility of the ferments of the saliva and pancreas to the action of acids and alkalies, that the atropine solution must be perfectly neutral. Several specimens of atropine sulphate that we have examined, have had a slight acid reaction.

In view of the apparent identity of the amylolytic ferments of the salivary and pancreatic secretions we have repeated in principle Stolnikow's experiment with human saliva, using *perfectly neutral* atropine sulphate.

To 10 c. c. of the dilute, neutral, saliva hitherto used, 0·3 gram of pure atropine sulphate was added (=3·0 per cent. of the alkaloid salt, while Stolnikow's mixture contained but 1·0 per cent.) and the solution allowed to stand for 18 hours at the Laboratory temperature. On now being added to the starch solution, diluted up to 100 c. c. [0·3 per cent. atropine sulphate] and placed at 40° C. for 30 minutes, the starch paste quickly became clear and it was found on examination that 29·16 per cent. of starch had been converted, while the control, in the presence of 0·2 per cent. of atropine sulphate, showed a conversion of 28·11 per cent. of the starch. Hence there had been no *destruction* of the salivary ferment by even 3·0 per cent. of pure atropine sulphate, although as our previous experiments show very much smaller percentages may, by their presence, *hinder the action of the ferment.*

Strychnine sulphate and brucine sulphate.

O. Nasse has previously studied the influence of 0·1 per cent. strychnine acetate on the diastatic action of saliva and has noted a slight increase in amylolytic action in the presence of the strychnine [109:100]. Our results with the two alkaloids are as follows:

Alkaloid.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1485 gram.	0·3028 gram.	27·25 per cent.
Strychnine sulphate.			
0·050 per cent.	0·1444	0·2936	26·42
0·250	0·1448	0·2936	26·42
0 500	0·1462	0·2976	26·78
Brucine sulphate.			
0·050 per cent.	0·1503	0·3060	27·54
0·500	0·1524	0·3100	27·90
1·000	0·1505	0 3060	27·54

With the brucine salt a slightly increased action is noticed in all three of the experiments; while with strychnine a constant diminution in amylolytic action is to be seen.

In this connection it is to be remembered, that a trace of free acid in the alkaloid salts would introduce an appreciable error into the results, and therefore all of the alkaloid salts experimented with, were especially purified for this purpose, any adhering acid being removed by repeated crystallization, etc.

The following table shows the relative acceleration and retardation of the various salts (the percentages more generally used) compared with their controls expressed as 100.

Table showing relative amylolytic action.

	0·0003 p. c.	0·0005 p. c.	0·001 p. c.	0·002 p. c.	0·005 p. c.	0·010 p. c.	0 025 p. c.	0·05 p. c.	0 1 p. c.	0 5 p. c.	1·0 p. c.	2·0 p. c.
l_2	94·3	50·8	38·2	16·7	0							
r_2		88·9	59·6	27·3								
l			97·1	91·2								
$N)_2$		106·2	111·7	95 9				77·6				
$)_4+5H_2O$		84·0		31·3		15·4	0					
$_2H_3O_2)_2+3H_2O$	100 7	100·3	100·3	97·7	85·2	85·7		99·5	98·6	88·9	78·5	
$)_3$	102·2	104·4	100 0	106·6	94·2							
sO_4		100·5	93·0		0							
$_4)_3AsO_4$		106·2	107·0		109·6		111·4	85·8	66·6	10·5	0	
$O)C_4H_4O_6$			104·5		107·9	114·5		114·5	120·2	168·3	101·6	79·4
$_2$	107·5		0									
$)_4+7H_2O$	99·7	101·3	98·5	96·3	90·9	84·5		52·8	47·5	0		
l_6		91·5		25·5		6·61	0					
$)_4+7H_2O$		83·2		106·3		109·8						
n_2O_8					68·5		0					
O_4+7H_2O							108·5			35·1		
N		86·9	72·2									
$e(CN)_6+3H_2O$							105·7		97·0			
$e_2(CN)_{12}$							107·0		91·3			
O_3										100·9	96·6	
O_3										104·8	106 0	
										105·6		
										104·7		
l										105·5	103·3	103·4
$B_4O_7+10H_2O$								0				
$)_2 . H_2SO_4+5H_2O$								113·7		129·4		114·9
$. H_2SO_4+7H_2O$								108·5		99·7		72·1
$_2 . H_2SO_4+2H_2O$								106·9		107·0		106·6
line)$_2 . H_2SO_4+3H_2O$								110·8		107·5		
$_2 . H_2SO_4$							98.2	94·0		94·5	99·1	83·5
$_2 . H_2SO_4+6H_2O$								96·9		98·2		
$_2 . H_2SO_4+H_2O$								101·0		102·4	101·0	

Influence of gases on the amylolytic action of saliva.

The well known analysis by Pfluger* of the gases of the submaxillary saliva have shown the presence of both oxygen and carbonic acid in this secretion; oxygen to the extent of 0·6 vol -per cent. and carbonic acid, by pump extraction, 22·5 vol.-per cent. It is, moreover, a well known fact that as the saliva flows into the mouth and becomes mixed with the food during mastication much air is absorbed. Do these three gases exert any influence on the amylolytic action of the ferment with which they are so constantly in contact?

Again, the amylolytic ferment of the pancreatic secretion, so near akin, it not identical with the salivary ferment, is subjected to the influence of the reducing gases of the intestinal canal, among which hydrogen may be present to the extent of 22·0 † vol.-per cent. and hydrogen sulphide in traces. What likewise is the effect of these two gases on amylolytic action?

The experiments were conducted as follows: 90 c. c. of diluted starch paste were placed in small, partially stoppered flasks and a stream of the gas allowed to pass through, until the fluid was thought to be saturated, then 10 c. c. of dilute saliva were added and the gas allowed to bubble through the solution for 30 minutes when the mixtures were boiled and the reducing bodies determined. Following are the results:

Gases.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch. converted.
0	0·1319 gram.	0 2684 gram.	24·15 per cent.
Air	0·1365	0·2780	25·02
Oxygen	0·1511	0·3080	27·72
Carbonic acid	0·1537	0·3136	28·22
Hydrogen sulphide	0·1377	0·2804	25.23
Hydrogen	0·1248	0·2540	22·86

It is interesting to see that air, oxygen and carbonic acid all stimulate and approximately in proportion to the extent in which they are present in the natural secretion, while of the reducing gases hydrogen retards and hydrogen sulphide stimulates.

The following table shows the relative acceleration and retardation of the several gases, compared with the control, expressed as 100.

Air	103·6
Oxygen	114·7
Carbonic acid	116·8
Hydrogen sulphide	104.4
Hydrogen	94·6

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* Physiologische Chemie, Hoppe-Seyler, p. 192.

† Maly in Hermann's Handbuch der Physiologie, vol. v, p. 25.

In accord with our results, Detmer* has found that the presence of carbonic acid invariably increases the amylolytic action of the diastase of malt. The same fact was previously observed by Baswitz.† O. Nasse,‡ however, has stated that the activity of ptyaline in human mixed saliva is not materially affected by oxygen, hydrogen or air. With carbonic acid, however, he noticed acceleration in amylolytic action.

Nature of the action of the metallic and other salts.

In what manner do the metallic and other salts act when they, by their presence, retard or completely stop the amylolytic action of saliva? Is it a process of gradual or sudden destruction of the ferment, or does the metallic salt combine with the ferment, forming a compound incapable of ferment action? or again, is the ferment mechanically thrown down with the precipitate of albumin or globulin produced by the addition of the metallic salt to saliva, or lastly does the salt by its mere presence introduce a condition unfavorable to the action of the ferment? All of these questions are interesting ones, and possibly all of them might be answered in the affirmative and be correct for some one or more of the substances experimented with.

It is obvious that the presence of 10 or 20 per cent. of such a salt as sodium chloride or potassium nitrate in a digestive mixture might retard the action of the ferment, since solutions so saturated, even with the products of digestion, do not admit of vigorous ferment action. But the larger number of metallic salts decidedly retard amylolytic action when present to the extent of only a few thousandths of one per cent., consequently their action must be of an entirely different nature. A number of these salts, such as mercuric chloride, are well known precipitants of albumin, but the saliva being so greatly diluted, in great part for this very reason, cannot yield sufficient precipitate with the mercury salt to mechanically precipitate the ferment. As a matter of fact, when the mercuric chloride solution is added to the diluted saliva, a very faint turbidity only, is produced. If now, some of the small percentages of mercuric chloride are added to the starch solution and then *larger quantities of saliva*, thus giving a larger amount of ferment together with a larger amount of accompanying albumin and globulin, what would be the effect on the amylolytic action of the ferment? Might we not expect, knowing that albumin and mercuric chloride readily combine, that the proteid matter present in the saliva, would serve as a shield to protect the ferment from the action of the mercury or other similar metallic salt? At the same time it might be supposed that, the ferment being left intact, any mercury-albumin compound formed might retard or destroy the ferment, though less energetically than the metallic salt alone.

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* Zeitschrift fur physiol. Chemie, vol. vii, p. 3.

† Berichte d. deutsch. chem. Gesell., vol. xi, p. 1443.

‡ Pflüger's Archiv, vol. xv, p. 471-481.

In an attempt to throw some light upon these points the following experiments were tried:

Action of mercuric chloride in the presence of larger amounts of ferment and proteid matter.

a. with 10 c. c. of original saliva.

$HgCl_2$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1772 gram.	0·3624 gram.	32·61 per cent.
0·0005 per cent.	0·1735	0·3548	31·93
0·0010	0·1695	0·3464	31·16

b. with 5 c. c. of original saliva.

0	0·1720 gram.	0·3516 gram.	31·64 per cent.
0·0005 per cent.	0·1340	0·2728	24·55

Comparing these results with those previously obtained with the same percentages of mercuric chloride, but with 2 c.c. of original saliva, we have:

$HgCl_2$	2 c.c. saliva.	5 c. c. saliva.	10 c. c. saliva.
0	44·28 per cent.	31·64 per cent.	32·61 per cent.
0·0005 per cent.	23·40	24·55	31·93
0·001	16·92	....	31·16

The intensity of action of the mercuric chloride, say 0·0005 per cent. in the three cases, varies greatly; thus with 2 c. c. of saliva the difference in the percentage of starch converted, between the control and the 0·0005 per cent. is 20·88, while with 5 c. c. of saliva the difference is 7·09 and with 10 c. c. of saliva only 0·68. Obviously then, the action of a given percentage of mercuric chloride can be considered as constant only for a given mixture or under definite conditions. Moreover, it would appear (in the 10 c. c.) that either the albuminous matter of the saliva has combined with all of the mercury, leaving the ferment free to act in a normal manner, except so far as it is impeded by the mercury-albumin compound, or else that only a small proportion of the ferment has been chemically precipitated, leaving an amount sufficient for energetic amylolytic action, since, as is well known, increase or decrease in the amount of ferment is not always followed by a proportionate change in the amount of reducing bodies formed. Of these two views the former is by far the most probable. Certainly the ferment is not mechanically precipitated by the formation of a mercury-albumin precipitate; if such were the case with 10 c.c. of saliva and 0·001 per cent. of mercuric chloride, decided retardation ought to have been observed.

Action of cupric sulphate in the presence of larger amounts of ferment and proteid matter.

$CuSO_4+5H_2O$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
	a. with 10 c.c saliva.		
0	0·1830 gram.	0·3748 gram.	33·73 per cent.
0·0005 per cent.	0·1775	0·3628	32·65
		Difference,	1·08
	b. with 5 c.c. saliva.		
0	0·1745 gram.	0·3568 gram.	32·11 per cent.
0·0005 per cent.	0·1640	0·3352	30·16
		Difference,	1·95
	c. with 2 c.c. saliva.		
0	0·1645 gram.	0·3360 gram.	30·24 per cent.
0·0005 per cent.	0·1140	0·2320	20·88
		Difference,	9·36

Action of zinc sulphate in the presence of larger amounts of ferment and proteid matter.

$ZnSO_4+7H_2O$	Wt. Cu in ¼	Total amount reducing bodies.	Starch converted.
	a. with 10 c.c. saliva.		
0	0·1830 gram.	0·3748 gram.	33·73 per cent.
0·05 per cent.	0·1737	0·3552	31·96
		Difference,	1·77
	b. with 5 c.c. saliva.		
0	0·1745 gram.	0·3568 gram.	32·11 per cent.
0·05 per cent.	0·1610	0·3288	29·59
		Difference,	2·52
	c. with 2 c.c. saliva.		
0	0·1645 gram.	0·3360 gram.	30·24 per cent.
0·05 per cent.	0·1320	0·2688	24.19
		Difference,	6·05

Glancing at the *differences* in these two series of experiments, we see that they accord with what was observed in the case of mercuric chloride, viz: that a given percentage of the metallic salt will produce a constant result only under definite conditions; increasing the proportion of albuminous matter diminishes, as in the case of the mercury salt, although not so greatly, the retarding action of the salt.

Evidently, the metallic salts do not act upon the ferment by their mere presence, for if such were the case the mere combination of the salt with the albumin present, would not so materially affect the result. If, on the other hand, they do act by combining with the ferment, forming it may be an insoluble compound or one incapable of ferment action, it is fair to presume that the combination would take place immediately upon mixing the two or very soon thereafter, and thus we should expect that the length of time the two stood in contact after the first few minutes, would have no effect on the amylolytic power of the mixture, while a gradual *destructive action* would be manifested by a gradual decrease of amylolytic power. With a view to testing this point we have tried the following experiment. Three mixtures were prepared as follows:

	A	*B*	*C.*
Saliva	2 c. c	2 c. c	2 c. c.
H_2O	8	7	8
$HgCl_2$ sol.	0	1	2
	10	10	10
Per cent $HgCl_2$	0	0·005	0·010

These were placed in a bath and warmed at 40° C. for 18 hours, after which 1 c. c. of the same mercuric chloride solution was added to *A* and then starch and water added to all three, making the volume in each case up to 100 c. c. The mixtures were then warmed at 40° C. for thirty minutes to test the activity of the ferment; *A* containing now 0·0005 per cent. mercuric chloride, *B* the same percentage and *C* 0·001 per cent.. In *A*, 19·8 per cent. of the starch was converted into reducing bodies, while in *B* and *C* there was no amylolytic action whatever. Thus by the previous action, for this length of time, of 0·005 per cent. mercuric chloride, the ferment was rendered incapable, on subsequent dilution, of exerting any diastatic action whatever.

Again, in a similar manner it was found that by warming the saliva for thirty minutes at 40° C. with 0·005 per cent. mercuric chloride and then adding starch paste and diluting to 100 c. c. so that the percentage of mercuric chloride was 0·0005, only 2·0 per cent. of the starch was converted, while the same quantity of saliva, in the presence of the same amount of mercury salt (0·0005 per cent.) converted 19·69 per cent. of starch.

Working with larger amounts of saliva, the following results were obtained:

	A.	*B*	*C*
Saliva	10 c. c	10 c c.	10 c c
H_2O	16	15	15
$HgCl_2$ sol.	0	1	1
	26	26	
Per cent. $HgCl_2$	0	0 002	

B was warmed at 40° C. for 15 minutes and *C* for 30 minutes; then 1 c. c. of the mercuric chloride solution was added to *A*, and all three diluted and mixed with starch paste. The three solutions were now exactly alike; all contained the same percentage of mercury salt (0·0005 per cent.) but *B* and *C* had been previously warmed with the salt for 15 and 30 minutes respectively. *A*, converted 31·32 per cent. of the starch, *B* 29·48 per cent. and *C* 27·97 per cent. Here we have what appears to be a gradual decrease in amylolytic power, but it does not seem sufficiently pronounced to account for the action of the mercury salt. It would appear rather, in this instance, as if the mercuric chloride exercised a selective action, combining with the proteid matter of the saliva, leaving the ferment free; but the mercury-proteid compound, being apparently possessed of some destructive action, exerts its influence, and thus the gradual decrease of amylolytic power noticed in *B* and *C*.

In the previous experiments, on the other hand, where *free* mercuric chloride is present, there not being sufficient albumin to combine with all of the mercury, there is apparently destructive action.

Experiments of like nature as the preceding, tried with cupric sulphate, gave the following results:

	A.	*B.*	*C.*	*D.*
Saliva	2 c. c	2 c. c.	2 c. c.	2 c. c
H_2O	18	17·8	17 8	17·8
$CuSO_4$ sol.	0	0·2	0·2	0·2
	20	20 0	20·0	20·0
Per cent $CuSO_4$	0	0·0005	0·0005	0·0005

B was warmed at 40° C. for 15 minutes, *C* for 30 minutes and *D* for 1 hour; 0·2 c. c. of the cupric sulphate solution was then added to *A* and lastly starch paste and water to 100 c. c. The amylolytic power of the four mixtures, expressed in the percentage of starch converted, was as follows:

A.	*B.*	*C.*	*D*
28·72	22·35	23·04	20·23

With zinc sulphate, somewhat similar results were obtained:

	A	*B.*	*C.*
Saliva	2 c. c.	2 c c.	2 c c.
H_2O	18	16	16
$ZnSO_4$ sol.	0	2	2
	20	20	20
Per cent. $ZnSO_4$	0	0 05	0·05

B was warmed at 40° C. for 30 minutes and *C* for 1 hour; then 2 c. c. of the zinc sulphate solution were added to *A*, and all three mixed with starch paste and water to 100 c. c. Each now contained 0·01 per cent. zinc sulphate and all three were then warmed at 40° C. for 30 minutes, to determine the activity of the ferment. *A*, converted 22·24 per cent. of the starch, *B* 11·88 per cent. and *C* 10·98 per cent.

These experiments would therefore indicate, on the part of the metallic salts experimented with, a destructive action towards the ferment, though loss of amylolytic power under the conditions of the experiments might also be due to more complete precipitation of the ferment in the more concentrated solution and under longer exposure to a temperature of 40° C. At the same time it is to be noticed, that any metallic-proteid compound formed with the above salts, has a far less destructive or retarding action than the free salt. Of these, the destructive action of mercuric chloride is most pronounced.

Potassium permanganate acts, doubtless, by direct destruction of the ferment through oxidation, while many of the alkali and alkali-earth salts produce their retarding effects by simple clogging of the digestive fluid; but the fact that 0·5 per cent. of one salt, as potassium antimony tartrate, for example, increases the amount of starch converted 68 per cent., and 0·5 per cent. of another salt, as magnesium sulphate, diminishes the amount of starch converted by 65 per cent., plainly indicates that there is something in the presence of these salts, dependent upon chemical constitution, that controls the action of the ferment.

VI.—INFLUENCE OF VARIOUS INORGANIC AND ALKALOID SALTS ON THE PROTEOLYTIC ACTION OF PEPSIN-HYDROCHLORIC ACID. BY R. H. CHITTENDEN AND S. E. ALLEN.

ALTHOUGH many experiments have been tried to ascertain the influence of various salts on ferment action since 1870, when Liebig* recorded the statement that the fermentative power of yeast is somewhat increased by a little potassium or sodium chloride, few systematic experiments, with a large variety of salts, have been made with the ferment of the gastric juice.

Alex. Schmidt† in 1876 studied the influence of sodium chloride on the digestive action of pepsin and hydrochloric acid. Wolberg‡ in 1880 studied, with the same ferment, the action of ammonium, potassium and sodium salts of nitric, hydrochloric and sulphuric acids and also the action of several alkaloids. Wernitz§ and also Petit‖ have studied the action of several metallic salts. Still later, Pfeiffer¶ has examined the influence of several alkali and alkali-earth salts on the digestive action of pepsin as well as of other ferments. Isolated experiments with single salts have likewise been recorded; these will be noticed later on.

It is thus seen that almost all work in this direction has been done with salts of the alkali and alkali-earth metals. No systematic attempt has been made to ascertain the influence on gastric digestion of the large number of metallic salts, in common use as poisons or therapeutic agents. With the exception of a few isolated cases, no accurate data are recorded bearing on this question. Observation has led to the belief that certain metallic salts interfere with digestion in the stomach, but few quantitative results are recorded to show the truth of such a belief.

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* Ueber Gährung, Quelle der Muskelkraft und Ernährung. Separatabdruck aus den Annalen der Chemie u. Pharmacie, 1870, p. 61.

† Pflüger's Archiv, vol. xiii, p. 97. Ueber die Beziehung des Kochsalzes zu einigen thierischen Fermentationsprocessen.

‡ Pflüger's Archiv, vol. xxii, p. 291. Ueber den Einfluss einiger Salze und Alkaloiden auf die Verdauung.

§ Quoted by Brunton. Pharmacology, p. 85–86.

‖ Études sur les ferments digestifs. Abstract in Jahresbericht für Thierchemie, 1880, p. 309.

¶ Ueber den Einfluss einige Salze auf verschiedene künstliche Verdauungsvorgänge. Abstract in Centralbl. med. Wiss., 1885, p. 328.

Our aim has been, therefore, to study more particularly the comparative influence on gastric digestion of various percentages of those salts, well known as poisons or therapeutic agents, which have hitherto been overlooked or but imperfectly studied. At the same time in order to make the work more complete, we have studied somewhat, the action of the alkali salts, experimented with by other observers.

Method employed.

The experiments were conducted in series, in which one of each series served as a control for comparison. The artificial gastric juice employed, was made from 0·2 per cent. hydrochloric acid and a glycerine extract of pepsin, in the proportion of 10 c. c. of the latter to 1 litre of the former. The volume of each digestive mixture was 50 c. c.; made up of 25 c. c. of the above-mentioned gastric juice and 25 c. c. of 0·2 per cent. hydrochloric acid, containing the salt to be experimented with. The material to be digested, consisted of purified and dried blood-fibrin, prepared by thorough washing with water, extraction with cold and boiling alcohol and lastly with ether. It was then ground to a coarse powder and dried at 100–110° C. 1 gram of the fibrin was used in each experiment. The digestive mixtures were warmed at 40° C. for two hours, then filtered upon weighed filters by the aid of pumps, the residue washed thoroughly with water, lastly with alcohol, and finally dried at 100–110° C. until of constant weight (48 hours). The amount of fibrin digested or dissolved, is a measure of the proteolytic action.

Cupric sulphate.

With this salt two series of experiments were made; one to ascertain the influence of small quantities, the other to show the effects of larger amounts of the substance.

$CuSO_4+5H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2854 gram.	71·46 per cent.	100·0
0·001 per cent	0·2508	74·92	104·8
0·005	0·2650	73·50	102·8
0·010	0·3067	69·33	97·0
0·025	0·3845	61·55	86·1
0·050	0·3877	61·23	85·6
0	0·2352	76·48	100·0
0·1	0·5315	46·85	61·2
0·3	0·7585	24·15	31·5
0·5	0·7976	20·24	26·4
0·8	0·8214	17·86	23·3
1·5	0·8480	15·20	19·8

The action of the salt is very marked; with even 0·010 per cent. there is a diminution in proteolytic action amounting to 3·0 per cent., while in the presence of 0·5 per cent. of the salt, there is retardation to the amount of nearly 75 per cent. The copper salt prevents almost entirely the swelling of the fibrin and doubtless its retarding action is due in part to this fact.

Lead acetate.

In view of the frequent cases of chronic poisoning with lead salts, the influence of the acetate on gastric digestion, seems especially interesting. The results, moreover, show decided action on the part of the salt; with small fractions of a per cent. pronounced increase in proteolytic action is to be noticed, while beyond 0·5 per cent. there is sudden and almost complete cessation of ferment action. In this respect, the salt acts very differently from the copper salt, with which a more gradual diminution is observed. The two largest percentages of the lead salt prevented entirely the swelling of the fibrin.

$Pb(C_2H_3O_2)_2+3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1936 gram.	80·64 per cent.	100·0
0·001 per cent.	0·1592	84·08	104·2
0·005	0·1892	81·08	100·5
0·010	0·1781	82·19	101·9
0·025	0·1691	83·09	103·0
0	0·2140	78·60	100·0
0·1	0·2310	76·90	97·8
0·3	0·4523	54·77	69·6
0·5	0·7419	25.81	32·8
0·8	0·9779	2·21	2·8
1·5	0·9938	0·62	0·7

Mercuric chloride.

This salt, which showed such a marked action on the amylolytic ferment of the saliva, causes a like diminution of proteolytic action in the case of pepsin; even with 0·001 per cent. there is retardation to the extent of over 6 per cent., calling the action of the control 100.

Petit* very erroneously states that mercuric chloride up to 0·4 per cent. does not hinder the action of pepsin.

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* Études sur les ferments digestifs. Abstract in Jahresbericht für Thierchemie, 1880, p. 309.

$HgCl_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·3759 gram	62·11 per cent.	100·0
0·001 per cent.	0·4140	58·60	93·8
0·005	0·1210	57·90	92·7
0	0·1307	86·93	100·0
0·1	0·1765	52·35	60·2
0·5	0·9007	9·93	11·1
1·0	1·0495	0	0

M. Marle* has previously experimented with mercuric chloride and has likewise found that small quantities of the salt exercise a retarding action upon gastric digestion; that as the percentage of corrosive sublimate is increased, the retarding action is correspondingly increased, although this effect is diminished up to a certain point, by increasing the strength of the digestive mixture. Marle considers that this action of mercuric chloride does not depend upon decomposition of the ferment nor upon a contraction of the albuminous matter, but rather that the salt in an acid solution enters into a chemical combination with the proteid matter and the latter is thus rendered impervious to the digestive action of the ferment.

In support of this view we offer the fact that fibrin introduced into an acid solution of pepsin in the presence of 1 per cent. of mercuric chloride, increases in weight; in the experiment given above to the extent of 49·5 milligrams. This would clearly indicate a combination of the two. Moreover, that mercuric chloride does not act by destroying the ferment we have ample proof, as the following experiment shows:

	A.	*B.*	*C.*
H_2O sol. glycerine pepsin	5 c. c.	5 c. c.	5 c. c
HCl (0·2 per cent.)	20	20	0
$HgCl_2$	0	0·025 gram.	0·025 gram.
H_2O	0	0	20 c. c.
	25	25 c. c.	25
Per cent. $HgCl_2$	0	0·1	0·1

These three mixtures were warmed at 40° C. for 24 hours; then to *A* was added 0·025 gram $HgCl_2$ dissolved in 25 c. c. 0·2 per cent. HCl, to *B* 25 c. c. 0·2 per cent. HCl and to *C* 25 c. c. 0·4 per cent. HCl. The three solutions were now exactly alike; in *B*, however, the ferment had been exposed to the action of 0·1 per cent. $HgCl_2$ in an acid solution for 24 hours, in *C* to the action of the same percentage of the mercury salt in an aqueous solution, while *A* served as a control. 1 gram of fibrin was added to each of the mixtures, which were then placed at 40° C. for 2 hours. *B* and *C* digested the same amount of fibrin as *A*, consequently the mercuric chloride could have exerted no destructive action whatever on the ferment.

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* Abstract in Jahresbericht fur Thierchemie, 1875, p. 168.

Wassilieff,* in Hoppe-Seyler's laboratory, found by comparative experiments that mercurous chloride (calomel) has no effect on the proteolytic action of pepsin.

Mercuric bromide, Mercuric iodide and Mercuric cyanide.

These three salts of mercury were experimented with, only so far as to compare the action of small quantities, with the action of like quantities of mercuric chloride. In using the bromide and iodide it was necessary, on account of their insolubility, to dissolve them with the aid of an equal weight of sodium chloride, consequently these two salts of mercury were doubtless present in the digestive mixtures, in part at least, as double salts. Marle, however, found that the action of mercuric chloride with small quantities of sodium chloride was not different from that of mercuric chloride alone, and doubtless the same is true of the iodide and bromide of mercury. Following are the results we obtained:

Mercury salt.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3590 gram.	64·10 per cent	100·0
$HgBr_2$			
0·005 per cent.	0·3731	62·69	97·8
0·025	0·3980	60·20	93·9
HgI_2			
0·005	0 3114	68·86	107·4
0·025	0·3904	60·96	95·1
$Hg(CN)_2$			
0·005	0·3105	68·95	107 5
0·025	0·3985	60·15	93·8
0·100	0·3183	68·17	106·3

From these it is evident that mercuric bromide is less vigorous in its hindering action than mercuric chloride; the iodide still less so, while mercuric cyanide, in similar percentages, appears to cause an increase in proteolytic action. The iodide, likewise, in the smallest percentage experimented with, causes increased proteolytic action. None of these salts then, approach mercuric chloride in the intensity of its hindering action on gastric digestion.

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* Ueber die Wirkung des Calomel auf Gährungsprozesse und das Leben von Mikroorganismen. Zeitschrift f. Physiologische Chemie, vol. vi, 113.

Stannous chloride.

This salt shows marked action in retarding gastric digestion; its retarding effect increasing directly with the amount of stannous chloride added.

$SnCl_2$.	Undigested residue.	Fibrin digested.	Relative pr lytic actio
0	0·2576 gram.	74·24 per cent.	100·0
0·025 per cent.	0·2728	72·72	97·5
0·1	0·4826	51·74	69·6
0·5	0·7332	26·68	35·9
1·0	0·8155	18·45	24·8
2·0	0·9010	9·90	13·3

Arsenious oxide.

This substance might naturally be expected, in view of its well known antiseptic properties, to hinder proteolytic action, more or less. It is known to hinder putrefaction and to prevent also the fermentative action of yeast. Contrary to our expectations, however, the action of arsenious oxide, so far as it is to be seen, is an accelerating one, causing increased proteolytic action. The following results were obtained:

As_2O_3.	Undigested residue.	Fibrin digested.	Relative pr lytic acti
0	0·2111 gram.	78·89 per cent.	100·0
0·05 per cent.	0·1872	81·28	103·0
0·1	0·2160	78·40	99·3
0·2	0·1900	81·00	102·6
0·5	0·1707	82·93	105·1

The stimulating action is slight, still it is plainly recognizable. Drs. Schäfer and Böhm* have previously studied the action of arsenious acid on the digestion of albumin by artificial gastric juice, and they came to the conclusion, using 0·02 and 0·04 gram As_2O_3 respectively, in 34 c. c. of fluid containing egg-albumin, that arsenious oxide is without influence on the decomposition of albumin by the gastric juice ferment. Our results, though not so large in number as theirs, would indicate a slight accelerating action.

Arsenic is known, when administered in small, repeated doses, to act as a tonic; the history of arsenic-eating, indicates that the substance has some positive tonic influence over nutrition, and Dr.

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* Jahresbericht für Theirchemie, 1872, p. 363. Ueber den Einfluss des Arsens auf die Wirkung der ungeformten Fermente.

Wood* states, "there is much reason for believing that it acts largely as a direct stimulant to nutrition." The results obtained in our experiments certainly accord with this statement.

Arsenic acid.

The experiments tried with arsenic acid, tend to confirm the accelerating action noticed with arsenious oxide. In the first series of experiments the following results were obtained:

H_3AsO_4.	Undigested residue.	Fibrin digested	Relative proteolytic action.
0	0·2696 gram.	73·04 per cent.	100·0
0·2 per cent.	0·2614	73·86	101·1
0·5	0·1514	84·86	116·1
2·0	0·2583	74·17	101·5
5·0	0·3915	60·85	83·3

The accelerating action is here so very pronounced, that a second series of experiments was undertaken by way of confirmation. These give in a general way the same results, although with 0·5 per cent. the stimulating action is not so pronounced as in the first experiment.

These two series of experiments illustrate another point, which it is well to mention here, namely: that definite percentages of any particular substance do not invariably give precisely the same result, even when compared with their respective controls. They do, however, generally point in the same direction, and although not always giving exactly the same numerical expression, they show clearly the nature and extent of the action.

H_3AsO_4.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2490 gram.	75·10 per cent.	100·0
0·2 per cent.	0·2401	75·99	101·2
0·5	0·2367	76·33	101·6
1·0	0·2335	76·65	102·0
2·0	0·2622	73·78	98·2
5·0	0·3176	68·24	90·8
0	0·1493	85·07	100·0
10·0	0·4207	57·93	68·1

Plainly then, arsenic acid in small percentages does accelerate the proteolytic action of pepsin-hydrochloric acid, while in large percentages (5–10) it causes a diminution in the action of the ferment. Arsenic acid tends to make the fibrin become very gelatinous.

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* Therapeutics, Materia Medica and Toxicology, p. 390.

Zinc sulphate.

With this salt, no experiments appear to have been hitherto made. Our results show a decided diminution in proteolytic action, even in the presence of 0·01 per cent. of the salt, while with a few thousandths of one per cent. the figures indicate a slight accelerating action. Three distinct experiments were made as follows:

$ZnSO_4+7H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1744 gram.	82·56 per cent.	100 0
0·001 per cent.	0·1609	83·91	101·6
0·005	0·1617	83·83	101·5
0·010	0·2053	79·46	96·2
0·025	0·2573	74·27	89·9
3·000	0·8400	16·00	19·3
0	0·1630	83·20	100 0
0·1	0·4848	51·52	61·9
0·3	0·7133	28·67	34 4
0·5	0·7382	26·18	31·4
0·8	0·7671	23·29	27·9
1·5	0·8202	17·98	21·6
0	0·1493	85·07	100·0
1·0	0·7683	23·17	27·2

A glance at these results, shows plainly a gradual decrease in proteolytic activity.

It is to be noticed that in the presence of the larger percentages of these metallic salts, the fibrin does not swell up in the 0·2 per cent. acid.

Manganous chloride.

In small fractions of one per cent. this salt gave such irregular results that it is doubtful if they can be relied upon as expressing any particular action. With 0·3 per cent. the retarding action of the manganese salt commences to be very pronounced. Following are the results:

$MnCl_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·1923 gram.	80·77 per cent.	100·0
0·001 per cent.	0·2022	79·78	98·7
0·010	0·1815	81·85	101·3
0·025	0·2066	79·34	98·2
0·050	0·1855	81·45	100·8
0	0·1880	81·20	100·0
0·3	0·3687	63·13	77·7
0·8	0·6438	35·62	43·8
1·5	0 6612	33·88	41·7
3·0	0·7400	26·00	32·0

Ferrous sulphate and Ferric chloride.

The salts of iron, doubtless on account of their physiological importance and their great therapeutic value, have been experimented with by several observers. It has been a prevalent opinion that iron salts tend to produce disturbances in gastric digestion. Petit,* however, states as a result of experiment, that preparations of iron, in small quantities, do not hinder the action of pepsin, but in large quantities they retard the action of the ferment, doing so according to Petit, by the hydrochloric acid of the gastric juice displacing the acid of the iron salt, thus forcing the pepsin to act with a less energetic acid. Düsterhoff,† dealing with the same question, came to the conclusion that iron salts of the organic acids, exercise the greatest retarding effect on pepsin digestion, and moreover, that ferrous salts are better adapted to the organism than ferric salts. Düsterhoff also concludes that while the retarding action of iron salts is doubtless due, in part, to the setting-free of the acid of the iron salt by the acid of the gastric juice, there is in addition a specific action of the iron preparation of an unknown nature, prejudicial to digestion. Lastly, Bubnow ‡ found that moist ferric hydroxide in small quantities (not weighed) causes a scarcely recognizable diminution in proteolytic action, while the presence of 1 per cent. of ferrous chloride and ferrous sulphate causes marked retardation, as does also an excess of ferric hydroxide. The most intense action was observed on the addition of 5 per cent. of ferrous sulphate. No quantitative results, that is, percentages of albumin digested were, however, obtained.

Our experiments were made only with crystallized ferrous sulphate and ferric chloride. It appears superfluous to try the action of ferric hydroxide, which must necessarily, if in sufficient quantity, neutralize the acid of the gastric juice and thus prevent digestion by withdrawal of the free acid.

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* Quoted by Bubnow in Zeitschrift für physiologische Chemie, vol. vii, p. 316; also abstract by Herter in Jahresbericht für Thierchemie, 1880, p. 309.

† Ueber den Einfluss von Eisenpräparaten auf die Magenverdauung. Jahresbericht für Thierchemie, 1882, p. 257.

‡ Ueber den Einfluss des Eisenoxyhydrats und der Eisenoxydulsalze auf künstliche Magenverdauung und Faulniss mit Pancreas. Zeitschrift für Physiologische Chemie, vol. vii, p. 315.

$FeSO_4+7H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1835 gram.	81·65	100·0
0·001 per cent.	0·1916	80·81	99·0
0·005	0·2241	77·59	95·0
0·010	0·1895	81·05	99·2
0·025	0·2573	74·27	90·9
0·050	0·2773	72·27	88·5
0	0·1935	80·65	100·0
0·1	0·3467	65·33	81·0
0·3	0·7274	27·26	33·8
0·8	0·8080	19·20	23·8
1·5	0·8447	15·53	19·2

Here, with the ferrous salt, we find pronounced diminution of proteolytic action, commencing even with 0·001 per cent. With ferric chloride, the following results were obtained.

Fe_2Cl_6.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1842 gram.	81·58 per cent.	100·0
0·001 per cent.	0·2111	78·89	96·7
0·005	0·2059	79·41	97·3
0·010	0·2165	78·35	96·0
0·050	0·2332	76·68	93·9
0	0·1961	80·39	100·0
0·3	0·6526	34·74	43·2
0·5	0·8035	19·65	24·4
0·8	0·8794	12·06	15·0
3·0	0·9582	4·18	5·2

A comparison of the two series of results, shows no pronounced and constant difference in the amount of action between the two iron salts; both retard proteolytic action about equally; although with the larger amounts, as with 0·5 per cent. and beyond, ferric chloride appears the most injurious. Comparing the results with those obtained with the manganese salt, which of late has been recommended as a therapeutic agent where iron cannot be taken, we see that the manganese is throughout, far less injurious than the two salts of iron.

As to the manner in which the iron salts produce their retarding effect on proteolytic action, it is evident that it cannot be due to a simple displacement of the acid of the iron salt, by which the pepsin is made to act with a less compatible acid, since ferric chloride acts similarly to the sulphate, in which case there could be no such injurious replacement.

Magnesium sulphate.

Pfeiffer* alone appears to have studied the influence of magnesium sulphate on gastric digestion. He found that retarding action commenced in the presence of 0·24 per cent. of the salt and was very great in the presence of 4·0 per cent. Our results show decided retarding action, even in the presence of 0·005 per cent. of the crystallized salt. At the same time, it is to be remembered throughout, that probably differences in the strength of gastric juice, would cause some variation in the amount of retardation, produced by any given percentage.

$MgSO_4+7H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1081 gram.	89·19 per cent.	100·0
0·005 per cent.	0·1910	80·90	90·7
0·010	0·2330	76·70	86·0
0·050	0·3260	67·40	75·5
0·100	0·4428	55·72	62·3
0	0·2605	73·95	100·0
0·3	0·7551	24·49	33·1
0·5	0·7886	21·14	28·5
0·8	0·8250	17·50	23·7
1·5	0·8891	11·09	15·0
3·0	0·8894	11·06	14·9

It is noticeable here, that while the retarding influence of the salt, becomes more and more pronounced as the percentage is increased, there comes a point (1·5 per cent.) when further addition does not materially influence the action of the ferment.

Potassium permanganate.

This salt, as with the amylolytic ferment of the saliva, shows very energetic action. Its influence is, without doubt, due to rapid oxidation and consequent destruction of the ferment; indeed, the (at first) bright red color of the solution became almost immediately bleached out and the solution, at the same time, completely deprived of proteolytic power. The following results testify to its extreme activity.

$K_2Mn_2O_8$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1951 gram.	80·48 per cent.	100·0
0·005 per cent.	0·8278	17·22	21·3
0·010	0·9949	0·51	0·6

It is thus more active in preventing proteolytic action, in gastric juice of the strength used, than in hindering the development of bacteria; although doubtless, the action of any one percentage is dependent in part, upon the amount of organic matter present. Marcus and Pinet* found that the permanganate in 0·1 per cent. would prevent the development of bacteria and in 1·5 per cent. would kill the fully developed organisms.

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* Abstract in Centralbl. med. Wiss., 1885, p. 328.

Potassium dichromate.

A single experiment with this salt gave the following results; showing a decided retarding action on gastric digestion.

$K_2Cr_2O_7$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2028 gram.	79·72 per cent.	100·0
0·01 per cent.	0·2476	75·24	94·4
0·10	0·6383	36·17	45·3

Potassium cyanide.

Potassium cyanide we found very active in diminishing the digestive power of pepsin; due in great part doubtless, to decomposition of the cyanide by the hydrochloric acid of the gastric juice with conseqent formation of pepsin-hydrocyanic acid. Our first results were as follows:

KCN.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·3255 gram.	67·45 per cent.	100·0
0·25 per cent.	0·9687	3·13	4·6
0·50	0·9912	0·88	1·3

Here, the fibrin did not swell at all, indicating the probable absence of free hydrochloric acid, although of course the potassium cyanide might, *per se*, prevent swelling.

With very much smaller percentages of cyanide, we obtained the following results:

KCN.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3098 gram.	69·02 per cent.	100·0
0·005 per cent.	0·4376	56·24	81·5
0·025	0·3750	62·50	90·5

Potassium ferrocyanide.

With this salt, the results are practically the same as with potassium cyanide; almost complete stopping of proteolytic action, even in the presence of small fractions of one per cent.

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* Action de quelques substances sur les bactéries de la putréfaction, Compt. rend. Soc. de Biolog., 1882, p. 718. Abstract in Jahresbericht für Thierchemie, 1882, p. 515.

$K_4Fe(CN)_6+3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3717 gram.	62·83 per cent.	100·0
0·05 per cent.	0·5969	40·31	64·1
0·10	0·7922	20·78	33·0
0·25	0·9585	4·15	6·6
0·5	1·0	0	0
0	0·3098	69·02	100·0
0·005	0·3562	64·38	93·3
0·025	0·3471	65·29	94·5

Potassium chlorate and Potassium nitrate.

These two oxidizing agents produce almost exactly the same effect on pepsin-hydrochloric acid digestion; a retarding action directly proportional to the amount of salt present.

$KClO_3$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2683 gram.	73·17 per cent.	100·0
0·3 per cent.	0·4565	54·35	74·3
0·8	0·7019	29·81	40·7
1·5	0·8173	18·27	25·0
3·0	0·8707	12·93	17·6
KNO_3.			
0	0·2870 gram.	71·30 per cent.	100·0
0·3 per cent.	0·5370	46·30	64·9
0·5	0·6247	37·53	52·6
0·8	0·7158	28·42	39·8
1·5	0·8148	18·52	25·9
3·0	0·8907	10·93	15·3

With potassium chlorate no experiments have been previously tried; with potassium nitrate, however, Wolberg[*] experimenting with quantities varying from 0·5 to 8·0 per cent. found in every instance, diminution in the proteolytic action of his pepsin solution. This, however, amounted to but little, except in the presence of 8 grams (8 per cent.) of the nitrate, where there was a diminution in the amount of fibrin digested, equal to 49·0 per cent. Even with 6 per cent. of the salt, Wolberg found after 24 hours, only a diminution of 6·8 per cent. in the fibrin digested.

In quantity, therefore, our results do not accord at all with Wolberg's, since as the table shows, even 0·3 per cent. of potassium nitrate caused a diminution in the quantity of fibrin digested, amounting to 35·1 per cent., when compared with the control (100); while the presence of 3 per cent. of the salt caused a diminution in proteolytic action amounting, in the quantity of fibrin digested, to nearly 85 per cent. The only apparent explanation of this difference in the results [unless due to difference in the amount of ferment] is in the length of time the mixtures were warmed at 40° C.; in Wolberg's 24 hours, in ours 2 hours. This, if the true reason of the difference, would imply on the part of the ferment, ability to gradually overcome the influence of small amounts of the substance and thus eventually to digest an equal quantity of proteid matter. This, however, would in turn imply that the object sought for, viz: the influence of different quantities or percentages of a substance on the action of the ferment is lost sight of. The length of time best adapted to the experiment, is naturally that which will bring out most clearly and decisively all *differences* of action.

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[*] Pflüger's Archiv, vol. xxii, p. 300. Ueber den Einfluss einiger Salze und Alkaloiden auf die Verdauung.

Sodium tetraborate (Borax) *and Boracic acid.*

Sternberg's experiments* with both of these substances, have shown that, although possessed of no germicide value, they prevent the multiplication of bacterial organisms and are thus valuable antiseptics.

Wolberg, in experiments made with artificial gastric juice, found that in a 24 hours digestion, 0·5 gram (0·5 per cent.) of borax caused a slight acceleration in proteolytic action (0·4 per cent.), while with 1 per cent. of the salt, retardation occurred to the extent of 23·3 per cent., and in the presence of 4·0 per cent. almost complete stopping of proteolytic action. Our results, however, fail to show any stimulating action on the part of the borate, although retardation is very pronounced.

$Na_2B_4O_7+10H_2O$.	Undigested residue.	Fibrin digested	Relative proteolytic action.
0	0·3610 gram.	63·90 per cent.	100·0
0·05 per cent.	0·3852	61·48	96·2
0·20	0·4080	59·20	92·6
0·5	0·7710	22·90	35·8
1·0	0·9899	1·01	1·5

Doubtless, the retarding action of this salt is due wholly to the liberation of boracic acid and the consequent neutralization of the hydrochloric acid of the gastric juice. Boracic acid itself, offers no obstacle to the proteolytic action of pepsin-hydrochloric acid; on the contrary it increases it, but pepsin-boracic acid has little digestive power. The influence of boracic acid on pepsin-hydrochloric acid is seen from the following experiments:

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* Amer. Jour. Med. Sciences, April, 1883, p. 335.

H_3BO_3.	Undigested residue.	Fibrin. digested.	Relative proteolytic action.
0	0·2395 gram.	76·05 per cent.	100·0
0·1 per cent.	0·2232	77·68	102·1
0	0·2049	79·51	100·0
0·5	0·1875	81·25	102·2
3·0	0·1729	82·71	104·2
6·0	0·1415	85·55	107·6

Evidently then, the action of borax consists simply in withdrawing from the pepsin the hydrochloric acid of the gastric juice. The low digestive power of pepsin and boracic acid is shown by the following experiment:

	A.	*B.*	*C.*	*D.*
H_2O sol. pepsin	50 c. c.	50 c. c.	50 c. c.	50 c. c.
HCl 0·2 per cent. ...	50	0	0	0
H_3BO_3	0	0·2 gram.	0·3 gram.	0·5 gram.
H_2O	0	50 c. c.	50 c. c.	50 c. c.
	100	100	100	100
	0·1 % HCl	0·2 % H_3BO_3	0·3 % H_3BO_3	0·5 % H_3BO_3

To each, was added 1 gram of purified fibrin, after which the mixtures were warmed at 40° C. for 2 hours. Following are the results:

	A.	*B.*	*C.*	*D.*
Wt. of undigested residue........	0·1180	0·9615	0·9705	0·9620
Per cent. digested	88·20	3·85	2·95	3·80

Ammonium oxalate.

With this salt the following results were obtained:

$(NH_4)_2C_2O_4+2H_2O$.	Undigested. residue.	Fibrin digested.	Relative proteolytic action.
0	0·3254 gram.	67·46 per cent.	100·0
0·010 per cent.	0·3579	64·20	95·1
0·025	0·3627	63·73	94·6
0·1	0·3920	60·80	90·1
0·5	0·9049	9·51	14·1
0	0·3098	69·02	100·0
1·0	0·9958	0·42	6·6

As to the cause of this retarding action, it is probable, that, as in the case of borax, the oxalic acid of the salt is displaced and the ferment compelled to act with the acid thus liberated. But if we compare the results of the present series with those of the preceding, we find that, with like percentages of the two salts, ammonium oxalate has far the greater retarding power, and yet it is well known that the ferment acts well when combined with oxalic acid. Moreover, our experiments show further on, that ammonium chloride has no greater retarding power than sodium chloride. Hence, there must be some reason, other than the one mentioned above, to account for all of the retarding action manifested by the oxalate.

Petit* states, that the maximum digestive action of oxalic acid, with 0·2–0·4 per cent. of his pepsin, is attained with 0·5–4·0 per cent. of the acid, according to the amount of pepsin.

The comparative digestive power of pepsin-oxalic acid and pepsin-hydrochloric acid is shown by the following experiments:†

	A.	*B.*	*C.*
H_2O sol. pepsin	50 c. c.	50 c. c.	50 c. c.
0·2 per cent. HCl	50	0	0
$C_2H_2O_4$	0	0·5 gram.	1·0 gram.
H_2O	0	50 c. c.	50 c. c.
	100	100	100
	0·1 % HCl	0·5 % $C_2H_2O_4$	1·0 % $C_2H_2O_4$

Warmed at 40° C. for 2 hours with 1 gram of pure, dry fibrin, the following results were obtained:

	A.	*B.*	*C.*
Wt. of undigested residue	0·2170	0·4450	0·4371
Per cent. digested	78·30	55·45	56·29

A second series, with larger amounts of oxalic acid, gave the following results:

	A.	B.	C.
H_2O sol. pepsin	50 c. c.	50 c. c.	50 c. c.
0·2 per cent. HCl	50	0	0
$C_2H_2O_4$	0	1·5 grams.	2·0 grams.
H_2O	0	50 c. c.	50 c. c.
	100	100	100
	0·1 per cent. HCl	1·5 per cent. $C_2H_2O_4$	2·0 per cent. $C_2H_2O_4$
Undigested residue	0·1085 gram.	0·3090 gram.	0·3640 gram.
Per cent. digested	89·15	69·10	63·60

These four results being placed on the same basis, i. e. compared with their respective controls (100) show as follows:

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* Jahresbericht für Thierchemie, 1880, p. 309.

† Made in this laboratory by Mr. R. W. Pinney.

Per cent. of acid.	Relative proteolytic action
0·1 HCl	100·0
0·5 $C_2H_2O_4$	70·7
1·0	71·7
1·5	77·5
2·0	71·3

This shows maximum action, with our amount of pepsin, in the presence of 1·5 per cent. of oxalic acid and shows, moreover, that the retarding influence of ammonium oxalate on proteolytic action, is not fully explained by the suggested neutralization of the hydrochloric acid of the gastric juice, unless it be that the ammonium chloride, formed by the reaction, effects pepsin-oxalic acid differently than it does pepsin-hydrochloric acid.

Sodium chloride.

With this salt we obtained, by our method, the following results:

NaCl.	Undigested residue.	Albumin digested.	Relative proteolytic acti
0	0·2936 gram.	70·64 per cent.	100·0
0·005 per cent.	0·2824	71·76	101·6
0·010	0·2896	71·04	100·5
0·025	0·3441	65·59	92·8
0·050	0·3511	64·89	91·8
0·100	0·3744	62·56	88·5
0	0·2669	73·31	100·0
0·3	0·4953	50·47	68·8
0·5	0·6175	38·25	52·1
0·8	0·6898	31·02	42·3
1·5	0·7825	21·75	29·6
3·0	0·8249	17·51	23·8

Alex. Schmidt* has recorded the retarding effect of sodium chloride on the proteolytic action of gastric juice; Petit† likewise, has stated that small quantities of sodium chloride interrupt the action of the ferment, and Wolberg‡ has recorded the same result with varying percentages of the salt; noting in addition, that very small quantities cause a slight acceleration of proteolytic action, amounting in his experiment to 2·5 per cent.

With 0·005 per cent. of the salt, we found as the results show, acceleration amounting to 1·6 per cent., larger amounts causing a gradual and proportional diminution in proteolytic action.

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* Pflüger's Archiv, xiii, p. 98.

† Jahresbericht fur Thierchemie, 1880, p. 309.

‡ Pflüger's Archiv, xxii, p. 298.

Pfeiffer* has also called attention to the retarding action of this substance.

Potassium chloride.

With this salt our results are as follows:

KCl	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·1552 gram.	84 48 per cent	100 0
0·005 per cent.	0·1817	81·83	96·8
0·025	0·1550	84·50	100·0
0·050	0·2565	74·35	88 0
0·100	0·2097	79 03	93·5
0	0·1930	80·70	100·0
0·3	0 3997	60·02	74·3
1·5	0·6815	31·85	39 4
3·0	0·7282	27·18	33·6

The only noticeable difference between the action of this salt and the preceding, is the absence of any acceleration on the part of the potassium chloride and with larger percentages, a less vigorous retarding action.

Ammonium chloride.

For the sake of comparison a few experiments were tried with this salt, with the following results:

$(NH_4)Cl$.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·1880 gram.	81·20 per cent.	100·0
0·3 per cent	0·1649	53·51	65 9
0·8	0·6615	33·85	41·7
3·0	0·6970	30·30	37·3

By looking at the table of comparisons, we see there is little constant difference in the amount of retardation caused by the ammonium, potassium and sodium salts of hydrochloric acid. This result, however, is quite different from that obtained by Wolberg, who found that ammonium chloride influenced proteolytic action but very little, while both potassium and sodium chloride caused great retardation. This difference in result, may be due to difference in strength of gastric juice or to difference in length of time the mixtures were warmed at 40° C.; certainly in our experiments, with pure anhydrous salts and 2 hours digestion, very little difference in digestive action is noticeable; throughout, sodium chloride causes a little less proteolytic action than the corresponding potassium salt, while of the ammonium salt, little is to be said except that it causes equal retardation.

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* Centralbl. med. Wiss., 1885, p. 328.

Wolberg in speaking of the relative retarding action of the three sodium salts of hydrochloric, nitric and sulphuric acids, states that the sulphate retards the most, then the nitrate and lastly the chloride. We have noticed the same fact in our work and take it as additional evidence of the liberation of the acid of the salt added, the retarding influence of the salt being dependent, in part, on the digestive power of the pepsin-*acid* formed.

The following experiments,* showing the relative digestive power of the several acids in conjunction with pepsin, testify to the probability of this view.

All of the solutions contained 50 c. c. of an aqueous solution of glycerine-pepsin, each having a total volume of 100 c. c. and differing from each other only in the nature and percentage of the acid present.

In testing the digestive power of the solution, 1 gram of pure fibrin was added and the mixtures warmed at 40° C. for two hours, after which the amount digested was determined in the usual manner.

Percentage of fibrin digested in the presence of the different percentages of acids.

	0·05 %	*0·1 %*	*0·2 %*	*0·3 %*	*0·4 %*	*0·5 %*	Control. *0·1 %* HCl
HNO_3†	9·20	48·75	73·65	67·20	46·00		87·65
H_2SO_4		19·50	24·70	25·80	22·55	24·95	88·05
$C_2H_4O_2$				3·70		2·30	87·50

Comparing these results with their respective controls, figured as 100, we have the following values for sulphuric and nitric acids, expressive of their relative proteolytic power when combined with pepsin, as compared with 0·1 per cent. hydrochloric acid under similar conditions.

Per cent. acid.	HNO_3.	H_2SO_4.
0·05	10·5	----
0·1	55·5	22·1
0·2	84·0	28·0
0·3	76·7	29·1
0·4	52·5	25·6
0·5	----	28·2

From this it is evident, that nitric acid is most active, with our amount of pepsin, in a 0·2 per cent. solution, while sulphuric acid attains its maximum action in 0·3 per cent.; moreover, nitric acid is more than four-fifths as active as 0·1 per cent. hydrochloric acid, while sulphuric acid is only a little more than one-fourth as active as the hydrochloric acid; acetic acid being practically worthless. Hence it is obvious, that, the base being the same, acetates, borates and other salts, the acids of which are not capable of working with pepsin, will most readily retard gastric digestion; then of the other salts experimented with, sulphates and lastly nitrates.

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* Made in this laboratory by Mr. R. W. Pinney.

† The acids are calculated as pure HNO_3, H_2SO_4, etc.

A glance at the table of comparisons, shows here and there, results which manifestly accord with this view, notably lead acetate, cupric sulphate, zinc sulphate,* magnesium sulphate and potassium nitrate.

That this withdrawal of free hydrochloric from the pepsin, is only a partial explanation of the mode of action of neutral salts is plain from the fact that chlorides exert very pronounced retarding action; moreover the action of these latter as well as of the others cannot be due merely to mechanical causes, viz: the semi-saturation of the digestive fluid, since 3·0 per cent. of boracic acid stimulates instead of retarding, and even the presence of 10 per cent. of arsenic acid causes retardation amounting to only 32 per cent. According to Petit,† moreover, saccharose to the extent of 16 per cent. does not interrupt gastric digestion. Again, that the base entering into the composition of the salt has much to do, in many cases, with its retarding proteolytic action, is apparent when we compare the action of ferric chloride, manganous chloride, sodium chloride, mercuric chloride and other metallic salts. That this action is due in part to capability of combining with the proteid matter, thus rendering it non-digestible, is unquestionable.

Acceleration, produced by neutral salts has been noticed by other observers, but no explanation of the cause has been offered. It seems probable, however, that in the case of pepsin-hydrochloric acid, one plausible reason, at least, may be suggested. If, as has been mentioned, many of the neutral salts are decomposed by the acid of the gastric juice, it would follow that the addition of very small amounts of the salts would diminish slightly the percentage of free hydrochloric acid, while the acid liberated from the salt, would be entirely without deleterious action. Nearly every experimenter in this direction has employed in the preparation of gastric juice 0·2 per cent. hydrochloric acid, which is well adapted for the purpose, but the action of the acid is dependent in part on the amount of ferment. With our solution of pepsin, the following results, expressed in the percentage of fibrin digested, were obtained with different percentages of hydrochloric acid; the amount of pepsin being the same in each case.

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* Compare Petit, Jahresbericht für Thierchemie. 1880, p. 309.

† Jahresbericht für Thierchemie, 1880, p. 309.

Per cent. HCl	*0·05*	*0·1*	*0·2*	*0·3*	*0·4*
Per cent. fibrin digested	73 8	*89·3*	81·0	81·7	63·8

This shows plainly, that, with the amount of pepsin employed in our experiments, acceleration of proteolytic action on the addition of neutral salts, might in some instances be due to slight diminution of free hydrochloric acid. That this is not the sole cause, however, is plain from the fact that closely related salts do not act alike.

Potassium bromide and Potassium iodide.

With these two potassium salts, the following results were obtained:

KBr.	Undigested residue.	Fibrin digested	Relative proteolytic action.
0	0·3837 gram.	61·63 per cent.	100·0
0·005 per cent.	0·3205	67·95	110·2
0·025	0·3035	69·65	113·0
0·10	0·4204	57·96	94·0
0·50	0·5690	43·10	70·0
1·00	0·6203	37·97	61 6
KI.			
0	0·2572 gram.	74·28 per cent.	100·0
0·005 per cent.	0 2755	72·45	97·5
0 025	0·3421	65·78	88·6
0·10	0·2841	71·59	96·4
0·50	0·6367	36·33	48 9
1·00	0·7586	24·11	32·5

By comparison of these two series of results, we see that there is a very decided difference in the action of the two salts; potassium bromide in very small quantity, causes a decided acceleration in proteolytic action, which is wholly lacking with potassium iodide. Again, there is a very great difference in the amount of retardation produced by the two salts; thus by comparison with their respective controls we see, that while 1 per cent. of potassium bromide causes retardation in digestive action, amounting to 38·4 per cent., the same percentage of iodide causes retardation amounting to 67·5 per cent. It is to be supposed, naturally, that in the presence of large amounts of the salts, the ferment must act wholly in combination with hydrobromic and hydriodic acid respectively; that is to say, the hydrochloric acid of the gastric juice must have replaced these two acids in the potassium salts, and thus new pepsin-compounds formed, of which pepsin-hydrobromic acid is the more active digestive agent.

Putzeys,* indeed, found that he obtained practically the same proteolytic action [retarding] with pepsin and hydriodic acid, as with potassium iodide and pepsin-hydrochloric acid. The following results, showing the relative digestive action of pepsin with hydrobromic and hydriodic acid respectively, were obtained by Putzeys.

HI	HBr.	Digestive products formed
0·625 gram.	----	15·86 per cent.
0·937	----	31·33
3·310	----	2·33
----	0·882 gram.	26·40
----	2·200	45·60
----	3·309	46·60

It is thus evident, that both hydrobromic and hydriodic acid can, to a certain extent, replace the hydrochloric acid of the gastric juice, although they are much less active than the latter acid. Moreover, hydrobromic acid is much more efficient than hydriodic in connection with the ferment, for in comparatively large doses the latter acid will completely stop proteolytic action. As a practical result, Putzeys suggests, that for therapeutic purposes, potassium bromide and iodide should be given ½ to 1 hour before meals. Our results are plainly in accord with Putzeys' observations.

The following table shows the relative influence on the proteolytic action of pepsin, of the various inorganic salts experimented with, compared with the controls, expressed as 100.

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* Jahresbericht fur Thierchemie, 1877, 279. De l'influence de l'iodure et du bromure de potassium sur la digestion stomacale.

Table showing relative proteolytic action.

	0·001 p. c.	0·005 p. c.	0·01 p. c.	0·025 p. c.	0·05 p. c.	0·1 p. c.	0·2 p. c.	0·3 p. c.	0·5 p. c.	0·8 p. c.	1·0 p. c.	1·5 p. c.	2·0 p. c.	3·0 p. c.	5·0 p. c.	10·0 p. c.
$HgCl_2$	93·8	92·7	----	----	----	60·2	----	----	11·4	----	0	----	----	----	----	----
$HgBr_2$	----	97·8	----	93·9	----	----	----	----	----	----	----	----	----	----	----	----
HgI_2	----	107·4	----	95·1	----	----	----	----	----	----	----	----	----	----	----	----
$Hg(CN)_2$	----	107·5	----	93·8	----	106·3	----	----	----	----	----	----	----	----	----	----
$CuSO_4 + 5H_2O$	104·8	102·8	97·0	86·1	85·6	61·2	----	31·5	26·4	23·3	----	19·8	----	----	----	----
$Pb(C_2H_3O_2)_2 + 3H_2O$	104·2	100·5	101·9	103.0	----	97·8	----	69·6	32·8	2·8	----	0·7	----	0	----	----
$SnCl_2$	----	----	----	97·5	----	69·6	----	----	35·9	----	24·8	----	13·3	----	----	----
As_2O_3	----	----	----	----	103·0	99·3	102·6	----	105·1	----	----	----	----	----	----	----
H_3AsO_4	----	----	----	----	----	----	101·2	----	101·6	----	102·0	----	98·2	----	90·8	68·1
$ZnSO_4 + 7H_2O$	101·6	101·5	96·2	89·9	----	61·9	----	34·4	31·4	27·9	27·2	21·6	----	19·3	----	----
Fe_2Cl_6	96·7	97·3	96·0	----	93·9	----	----	43·2	24·4	15·0	----	----	----	5·2	----	----
$FeSO_4 + 7H_2O$	99·0	95·0	99·2	90·9	88·5	81.0	----	33·8	----	23·8	----	19·2	----	----	----	----
$MnCl_2$	98·7	----	101·3	98·2	100·8	----	----	77·7	----	43·8	----	41·7	----	32·0	----	----
$MgSO_4 + 7H_2O$	----	90·7	86·0	----	75·5	62·3	----	33·1	28·5	23·7	----	15·0	----	14·9	----	----
$K_2Mn_2O_8$	----	21·3	0·6	----	----	----	----	----	----	----	----	----	----	----	----	----
$K_2Cr_2O_7$	----	----	94·4	----	----	45·3	----	----	----	----	----	----	----	----	----	----
KCN	----	81·5	----	90·5	----	----	----	----	1·3	----	----	----	----	----	----	----
$K_4Fe(CN)_6 + 3H_2O$	----	93·3	----	94·5	64·1	33·0	----	----	0	----	----	----	----	----	----	----
$(NH_4)_2C_2O_4 + 2H_2O$	----	----	95·1	94.6	----	90·1	----	----	14·1	----	0·6	----	----	----	----	----
$Na_2B_4O_7 + 10H_2O$	----	----	----	----	96·2	----	92·6	----	35·8	----	1·5	----	----	----	----	----
H_3BO_3	----	----	----	----	----	102·1	----	----	102·2	----	----	----	----	104·2	----	----
$KClO_3$	----	----	----	----	----	----	----	74·3	----	40·7	----	25·0	----	17·6	----	----
KNO_3	----	----	----	----	----	----	----	64·9	52·6	39·8	----	25·9	----	15·3	----	----
KCl	----	96·8	----	100·0	88·0	93·5	----	74·3	----	----	----	39·4	----	33·6	----	----
NaCl	----	101·6	100·5	92·8	91·8	88·5	----	68·8	52·1	42·3	----	29·6	----	23·8	----	----
$(NH_4)Cl$	----	----	----	----	----	----	----	65·9	----	41·7	----	----	----	37·3	----	----
KBr	----	110·2	----	113·0	----	94·0	----	----	70·0	----	61·6	----	----	----	----	----
KI	----	97·5	----	88·6	----	96·4	----	----	48·9	----	32·5	----	----	----	----	----

Alkaloid salts.

According to Petit, alkaloids do not interrupt the action of pepsin in acid solution. Wolberg, however, found that morphine chloride, strychnine, quinine sulphate and narcotine, used in very small quantities (0·5 grain to 100 c. c.), caused in every instance, excepting with quinine sulphate, retardation amounting on an average to nearly 3 per cent.; with quinine sulphate, slight acceleration was obtained. Strychnine and narcotine, in very small quantity, gave slight acceleration. Dr. H. v. Boeck* states, that quinine is without influence on the activity of pepsin.

In our experiments, using larger amounts of the alkaloid salts, retardation is very pronounced, doubtless due in part to replacement of the sulphuric acid of the salts by the acid of the gastric juice.

Alkaloid salt.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3555 gram.	64·45 per cent.	100·0
$(Sr)_2 . H_2SO_4 + 6H_2O$.			
0·5 per cent.	0·6825	31·75	49·2
1·0	0·8213	17·87	27·7
$(Br)_2 . H_2SO_4 + H_2O$.			
0·5	0 5685	43·15	66·9
1·0	0·7700	23·00	35·6
$(At)_2 . H_2SO_4$.			
0·5	0·6412	35·88	55·6
$(Q)_2 . H_2SO_4 + 7H_2O$.			
0·5	0·6606	33·94	52·6
$(Ci)_2 + H_2SO_4 + 2H_2O$.			
0·5	0·6885	31·15	48·3
$(Mo)_2 . H_2SO_4 + 5H_2O$.			
0·5	0·6225	37·75	58·5
$(Na)_2 . H_2SO_4 + H_2O$.			
0·5	0·6365	36·35	56·4

Percentage retardation of the alkaloid salts (0·5 *per cent.*)

Strychnine	50·8
Brucine	33·1
Atropine	44·4
Quinine	47·4
Cinchonine	51·7
Morphine	41·5
Narcotine	43·6

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* Zeitschrift für Biologie, vol. vii, p. 428.

VII.—INFLUENCE OF VARIOUS THERAPEUTIC AND TOXIC SUBSTANCES ON THE PROTEOLYTIC ACTION OF THE PANCREATIC FERMENT. BY R. H. CHITTENDEN AND GEO. W. CUMMINS, PH.B.

WITH the proteolytic ferment of the pancreatic juice, few systematic experiments have been tried to ascertain the influence of those substances the frequency of whose use, as therapeutic or toxic agents, renders their action on this ferment a matter of no small consequence. The well known experiments of Heidenhain, Kühne and Schmidt have been confined to the action of sodium carbonate, sodium chloride and kindred salts of physiological importance. Pfeiffer* has indeed, in addition, experimented with a few of the sulphates of the alkalies and alkali-earths, but of the large number of metallic and other salts in common use, few have been tried, while the action of alkaloids and the gases occurring in the intestinal canal, has not been studied at all.

Method employed.

It is a characteristic of the proteolytic ferment of the pancreatic juice, that it exercises considerable digestive power in a *neutral* solution.† In view therefore of the fact that the ferment, while in the intestinal canal, may be obliged to act in an acid-reacting medium (due to acid-proteids) as frequently as in an alkaline or neutral one, it seemed advisable in the experiments, to employ a neutral solution of trypsin. Moreover, it becomes necessary to use such a solution, if salts of the heavy metals are to be experimented with, since in alkaline solutions, carbonates or oxides of the metals would be formed and thus affect the results; still, again, the action of all substances can be best studied in neutral solution, since under such conditions, no replacements or decompositions of any kind take place to complicate the action of the substance, other than the direct action on the ferment or the proteid matter. Hence, a neutral solution has in every case been employed as being the most satisfactory, while only such substances have been experimented with, as contained no free acid or alkali, the destructive action of which is well known.

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* Centralbl. med. Wiss., 1885, p. 328.

† See Chittenden and Cummins, Amer. Chem. Jour., vol. vii, p. 46. Also Trans. Conn. Acad., vol. vii.

The solution of trypsin was prepared according to Kuhne's* method from dried ox pancreas freed from fat; 40 grams dry pancreas in 500 c. c. 0·1 per cent. salicylic acid, neutralized and diluted to 2 litres.† In each digestion experiment, 25 c. c. of this neutral trypsin solution were used, to which was added 25 c. c. of water containing the substance to be experimented with, or in the control 25 c. c. of water alone, making the volume of the digestive mixture in each case 50 c. c. In testing the proteolytic action of the different solutions, 1 gram of pure dry, pulverized fibrin, described in the preceding article, was added and the mixtures warmed at 40° C. for six hours, after which the undigested fibrin was filtered on weighed filters, washed thoroughly and dried at 100–110° C. until of constant weight.

In this work as in the study of the salivary ptyalin and pepsin, it has been the effort mainly to ascertain the relative action of small quantities of the various salts, rather than the percentages necessary to completely stop the action of the ferment, this to our mind being much the more important.

Mercuric chloride.

With small percentages of this salt, the following results were obtained:

$HgCl_2$.	Undigested residue	Fibrin digested.	Relative proteolytic action.
0	0·4495 gram.	55·05 per cent	100·0
0·002 per cent	0·4465	55·35	100·5
0·003	0·4405	55·95	101·6
0·005	0·4562	54·38	98·7
0·025	0·5076	49·24	89·4
0·100	0·7753	22·47	40·8

As seen from the table, 0·1 per cent. mercuric chloride diminishes the proteolytic action of the ferment more than one-half. Its action in this percentage is more energetic than on pepsin, although the smaller quantities are proportionally far less active; in one case (0·003 per cent.) even causing acceleration.

Wassilieff‡ has studied the influence of mercurous chloride on pancreatic digestion and finds that calomel does not affect the action of the proteolytic ferment, while it does prevent the formation of putrefaction products, viz: indol, phenol, etc.

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* Untersuchungen aus der physiolog. Inst. d. Universitat Heidelberg, vol. i, p. 222.

† The solution was kept from putrefaction by the use of a little 20 per cent. alcoholic solution of thymol; enough of which remained dissolved in the fluid to prevent putrefaction also during the six hours digestion at 40° C.

‡ Zeitschrift fur physiol. Chemie, vol. vi, p 112.

Mercuric iodide and Mercuric bromide.

These salts dissolved in sodium chloride in such proportion that the ultimate solutions contained like percentages of both salts, gave the following results:

HgI_2.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4707 gram.	52·93 per cent.	100·0
0·005 per cent.	0·4780	52·20	98·6
0·025	0·5085	49·15	92·8
0·100	0·5994	40·06	75·6
0·200	0·6580	34·20	64·6
$HgBr_2$.			
0	0·4707 gram.	52·93 per cent.	100·0
0·005 per cent	0·4400	56·00	105·8
0·025	0·4840	51·60	97·4
0·100	0·5721	42·79	80·8
0·200	0·6548	34·52	65·2

Aside from a slight acceleration, noticed with 0·005 per cent. of bromide, the two salts act very much alike, causing retardation of proteolytic action; less pronounced however, than that caused by mercuric chloride.

Mercuric cyanide.

The action of this salt is somewhat peculiar, causing at first when in small quantity, noticeable retardation followed in the presence of larger percentages by increased proteolytic action, though still below the action of the normal solution of trypsin.

In a general way, its action on this ferment accords very nearly with its action on the amylolytic ferment of saliva and the proteolytic ferment of the gastric juice.

$Hg(CN)_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2668 gram.	73·32 per cent.	100·0
0·005 per cent.	0·3192	68·08	92·8
0·025	0·3209	67·91	92·6
0·050	0·3244	67·56	92·1
0·100	0·3308	66·92	91·2
0	0·3675 gram.	63·25 per cent.	100·0
0·3 per cent.	0·4094	59·06	93·3
0·5	0·3880	61·20	96·7
1·5	0·3932	60·68	95·9

Its action is to be attributed mainly to the hydrocyanic acid radical, judging from the action of potassium cyanide on the one hand and the action of mercury salts on the other. There is, however, without doubt a close connection between the action of these salts and their power of combining with proteid matter in general.

Cupric sulphate.

With this salt a more energetic retarding action is to be noticed, than in the case of the proteolytic ferment of gastric juice; 0·1 per cent. of the salt causing a retardation in proteolytic action of 65·7 per cent. as compared with a retardation of 38·8 per cent. in the case of pepsin.

$CuSO_4+5H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·5035 gram.	49·65 per cent.	100 0
0·005 per cent.	0·5027	49·73	100·1
0·025	0·5320	46·80	94·2
0·050	0·5856	41·44	83·4
0·100	0·8295	17·05	34 3

Lead acetate.

With this salt the following results were obtained, agreeing essentially in the smaller percentages, with those obtained with cupric sulphate. In the presence of 0·1 per cent., however, retardation is far less than with the same percentage of cupric sulphate; 0·5 per cent. completely stops proteolytic action.

$Pb(C_2H_3O_2)_2+3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4562 gram.	54·38 per cent.	100·0
0·005 per cent.	0·4655	53·45	98·2
0 025	0·5391	46·09	84·7
0·050	0·5660	43·40	79·8
0·100	0·6410	35 90	66·0
0·500	1·0	0	0

Stannous chloride.

This salt, in conformity with its action on the amylolytic ferment of saliva, causes very decided retardation in the proteolytic action of trypsin, requiring but a very small amount of the salt to completely stop the action of the ferment.

$SnCl_2$.	residue.	digested.	lytic action.
0	0·3875 gram.	61·25 per cent.	100·0
0·0005 per cent.	0 4495	55·05	89·8
0·005	0·5370	46·30	75·5
0·025	1·0	0	0

Arsenious oxide.

Schäfer and Böhm have experimented with arsenious acid, studying its influence on the proteolytic action of a glycerine infusion of

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[*] Abstract in Jahresbericht für Thierchemie, 1872, p. 363.

pancreas. They find that arsenious acid is without influence on the action of the ferment. We do not know whether they added arsenious acid to a neutral or alkaline solution of the ferment, as we have seen only an abstract of their paper, but we find that the addition of very small amounts of arsenious oxide to a neutral solution of trypsin, does produce a slight retardation in the proteolytic action of the ferment. Owing to the comparative insolubility of arsenious oxide in water, only small percentages could be employed, but these show, as might be expected from the acid character of the substance, a decrease in proteolytic action.

As_2O_3.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·4598 gram.	54·02 per cent.	100·0
0·001 per cent.	0·4630	53·70	99·1
0·005	0·4513	54·87	101·5
0 025	0·4710	52·90	97·9
0·050	0·4723	52·77	97·6

Arsenic acid.

This substance, in conformity with its more decided acid character, causes greater retardation than arsenious acid; which fact tends, by analogy, to make the retarding action of the latter still more probable. The results are as follows:

H_3AsO_4.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4598 gram.	54·02 per cent.	100·0
0·005 per cent.	0·4563	54·37	100·6
0·025	0·4887	51·13	94·6
0·050	0·5264	47 36	87·4
0·100	0·6340	36·60	67·7

Ammonium arsenate.

This salt in small amount, appears to increase the proteolytic action of the ferment, which fact would indicate that the retarding action of the two preceding arsenic compounds is due more to their acid character than to the arsenic contained in them.

$(NH_4)_3AsO_4$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4598 gram.	54·02 per cent.	100·0
0·050 per cent.	0·4620	53·80	99·6
0·100	0·4442	55·58	102·8
0	0·3675	63·25	100·0
0·5	0·4071	59·29	93·7

Potassium antimony tartrate.

This compound of antimony fails to produce with trypsin the marked acceleration, noticed with the amylolytic ferment studied.

This difference in action, since both ferments were in neutral solution, indicates a specific difference in the nature of the two ferments. Following are the results obtained:

$K(SbO)C_4H_4O_6$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3978 gram.	60·22 per cent.	100·0
0·2 per cent.	0·4105	58·95	97·8
0·5	0·4129	58·71	97·4
1·0	0·4258	57·42	95·3
1·5	0·5406	45·94	76·2

Ferric chloride and ferrous sulphate.

With these two salts of iron the following results were obtained:

Fe_2Cl_6.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·5215 gram	47·85 per cent.	100·0
0·005 per cent.	0·5431	45·69	95·4
0·025	0·6243	37·57	78·5
0·050	0·7457	25·43	53·1
0·100	1·0	0	0
$FeSO_4+7H_2O$.			
0	0·4075 gram.	59·25 per cent.	100·0
0·005 per cent.	0·4548	54·52	92·0
0·05	0·6225	37·75	63·7
0·10	0·7177	28·23	47·6
0·25	0·7104	28·96	48·8
0·50	0·7219	27·81	46 9
1·00	0·7675	23·25	39·2
1·50	0·7861	21·39	36·1

Ferric chloride is seen to be far more energetic in its hindering action on the proteolytic ferment, than the ferrous salt. A like result was obtained with the amylolytic ferment of the saliva and to a lesser extent with pepsin-hydrochloric acid.

Bubnow* has tried experiments with both of these salts and found, as might be expected, that when present to the extent of 5 per cent. they prevented the appearance of putrefaction products (indol, phenol, etc.) but did not interfere with the action of the unorganized ferment. As no quantitative results were obtained, we can make no direct comparisons. In our experiments, it is to be remembered that putrefaction is prevented by thymol.

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* Zeitschrift für physiol. Chemie., vol. vii, p. 327.

Manganous chloride.

Following are the results obtained with this salt:

$MnCl_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·5215 gram.	17·85 per cent	100·0
0·005 per cent.	0·5201	47·99	100·3
0·050	0·5139	48·61	101·6
0·100	0·5483	45·17	94·4
0·500	0·6807	31·93	66·7

Comparing the influence of this salt on the proteolytic action of trypsin, with the results obtained with the closely related iron salts, it is seen that the manganese salt has a far less retarding influence than the latter; a fact which agrees with what was observed in studying its action on pepsin-hydrochloric acid.

Consequently, manganese salts, in so far as they are adapted to replace iron salts as therapeutic agents, are apparently less liable to interfere with normal digestive action.

Zinc sulphate.

$ZnSO_4+7H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·5035 gram.	49·65 per cent.	100·0
0·005 per cent.	0·5175	48·25	97·2
0·025	0·5805	41·95	84·5
0·050	0·6802	31·98	64·4
0·100	0·8301	16·99	34·2

The action of this salt on trypsin is, both in character and extent, similar to its action on the amylolytic ferment of saliva. Compared with pepsin-hydrochloric acid, however, the action of the salt is seen to be more energetic on the pancreatic ferment.

Barium chloride.

The following results were obtained:

$BaCl_2+2H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3710 gram.	62·90 per cent	100·0
0·05 per cent.	0·3515	64·85	103·1
0·5	0·3885	61·15	97·2
3·0	0·4986	50·14	79·7

With this salt a slight acceleration in proteolytic action is to be seen with 0·05 per cent., while the larger amounts cause noticeable retardation.

Magnesium sulphate.

$MgSO_4+7H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3495 gram.	65·05 per cent.	100·0
0·05 per cent.	0·3541	64·59	99·3
0·5	0·3810	61·90	95·1
3·0	0·4706	52·94	81·3

Here retarding action is similar in extent to the action of barium chloride. Compared with zinc sulphate, the difference in action on trypsin is about the same in extent as the difference found in the action of the two salts on the amylolytic ferment of saliva. The retarding action of this salt on pancreatic digestion has been previously noticed by Pfeiffer.*

Potassium permanganate.

The retarding action of potassium permanganate is very pronounced. There is, however, a noticeable difference between the action of the salt on trypsin and its action on pepsin and ptyalin, the two latter being much more readily affected by the permanganate; that is, by much smaller percentages.

$K_2Mn_2O_8$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4562 gram.	54·38 per cent.	100·0
0·001 per cent.	0·4570	54·30	99·8
0·003	0·4608	53·92	99·1
0·010	0·4833	51·67	95·0
0	0·3675	63·25	100·0
0·1	0·4487	55·13	87·1
0·5	0·7769	22·31	35·2
1·0	1·0	0	0

Potassium dichromate.

$K_2Cr_2O_7$.	Undigested residue.	Fibrin digested	Relative proteolytic action.
0	0·3978 gram.	60·22 per cent.	100·0
0·05 per cent.	0·4032	59·68	99·1
0·2	0·4245	57·55	95·5
0·5	0·4693	53·07	88·1
1·0	0·5525	44·75	74·3
1·5	0·6321	36·79	61·0

The retarding action of this salt is proportionally greater than that of the other potassium salts, indicating that the acid has a specific action of its own. Moreover, being an acid salt, the acid radical is present in larger amount than in the neutral potassium salts experimented with.

Potassium cyanide.

The very pronounced acceleration in proteolytic action caused by the larger percentages of this salt is very interesting, especially when we recall the fact, that the retarding action of the same salt on the amylolytic ferment of saliva and on the proteolytic ferment of gastric juice was equally decided, even with very small pecentages.

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* Centralbl med. Wiss., 1885, p. 328.

KCN.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·2668 gram.	73·32 per cent	100·0
0·005 per cent.	0·2790	72·10	98·3
0·025	0·2840	71·60	97 6
0·050	0·2678	73·22	99·8
0·100	0·2600	74 00	100 9
0	0·3675	63·25	100·0
0·3	0 2076	79·24	125·2
0·5	0·1985	80·15	126·7
1·0	0·2697*	73·03	115·4
1·5	0·3315*	66·85	105·6

Potassium ferrocyanide and Potassium ferricyanide.

With these two salts the following results were obtained:

$K_4Fe(CN)_6+8H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·3045 gram.	69·55 per cent.	100·0
0·005 per cent.	0·3363	66·37	95·4
0·50	0·3293	67·07	96·4
2·00	0·3753	62·47	89·8
$K_6Fe_2(CN)_{12}$			
0·005 per cent.	0·3268	67·32	96·8
0·05	0·3370	66·30	95·3
2·00	0·3912	60·88	87·5

Here, unlike the cyanide, there is no acceleration in the proteolytic action of the ferment, neither on the other hand is there very pronounced retardation; less indeed than is produced by a like percentage (2·0) of sodium chloride.

Sodium tetraborate.

With this salt we obtained the following results:

$Na_2B_4O_7+10H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·4116 gram.	58·84 per cent.	100·0
0·05 per cent.	0·3729	62·71	106·5
0·2	0·3260	67 40	114·5
0·5	0·2332	76·68	130·3
1·0	0·1860	81·40	138·3
2·0	0·1663	83·37	141.7
3·0	0·2276	77·24	131·2
5·0	0·3141	68·59	116·5

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* It was impossible to wash these completely, consequently the weights are undoubtedly too high.

Here, unlike the action of the salt on the two preceding ferments studied, there is to be seen a gradual increase in proteolytic action, which is very pronounced in the presence of 2 per cent. of the salt, then gradually diminishes; although even with 5 per cent. of the crystallized salt, the action of the ferment continues to be increased far above that of the normal juice.

Sodium sulphate.

Following are the results obtained:

$Na_2SO_4+10H_2O$	residue.	digested.	Relative proteolytic action
0	0·3495 gram.	65·05 per cent.	100·0
0·05 per cent.	0·3602	63·98	98·3
0·5	0·3558	64·42	99·0
2·0	0·3938	60·62	93·1
5·0	0·4360	56·40	86·7

The retarding action of this salt is quite pronounced, although it is noticeable that its retarding action is not equal to that of magnesium sulphate nor indeed to that of sodium chloride. Pfeiffer* has also noticed the retarding action of this salt. Weiss,† however, has stated that sodium sulphate in very small quantity increases the proteolytic activity of a pancreas extract. As we have not seen the original article we do not know whether definite percentages were employed or not; with 0·05 per cent. of the crystallized salt, under the conditions of our experiment, there was certainly no acceleration in proteolytic action.

Potassium chlorate and Potassium nitrate.

$KClO_3$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3662 gram.	63·38 per cent.	100·0
0·05 per cent.	0·3991	60·09	94·8
0·50	0·3743	62·57	98·7
2·00	0·4101	58·99	93·0
KNO_3.			
0	0·3710 gram.	62·90 per cent.	100·0
0·05 per cent.	0·3751	62·49	99·3
0·5	0·3804	61·96	98·5
2·0	0·4080	59·20	94·1
5·0	0·4473	55·27	87·8

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* Loc. cit.

† Jahresbericht für Thierchemie, 1876, p. 177.

The effects of these two salts on the proteolytic action of the ferment are very similar, with the exception that potassium chlorate appears to be a little more energetic in action than the nitrate. Weiss* has stated that a very small quantity of potassium nitrate added to a pancreas infusion, causes slight increase in the proteolytic activity of the ferment. This, however, is not observable in our experiment with 0·05 per cent., although retardation is very slight.

Potassium chloride.

This salt, unlike sodium chloride, appears to cause retardation of proteolytic action, and is without any accelerating influence whatever. The extent of its retardation, however, is not so great as in the case of sodium chloride. Following are the results obtained.

KCl.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3662 gram.	63·38 per cent.	100·0
0·05 per cent.	0·3740	62·60	98·7
0·5	0·3672	63 27	99·8
2·0	0·4331	56·69	89·4
5·0	0·4723	52·77	83·2

Sodium chloride.

With this salt many previous experiments have been tried; Heidenhain,† after noting the accelerating action of sodium carbonate, tried the influence of sodium chloride and found that it increased the proteolytic power of the ferment. E. Pfeiffer, however, states that he found sodium chloride to exert a retarding influence on the proteolytic action of the pancreatic ferment. Pfeiffer‡ moreover states that he found sodium carbonate to exert its accelerating action only when present in 0·5 per cent., not in 0·24 per cent. as found by Heidenhain. Recent results§ obtained by us in another connection, however, fully substantiate Heidenhain's statements on this point. Lastly, Lindberger‖ has found that sodium chloride, which in a neutral or alkaline solution of trypsin accelerates its proteolytic action, causes in an acid solution (0·018 per cent. HCl, presumably as acid-proteid) marked retardation of ferment action, without, however, causing destruction of the ferment, as after dialysis the ferment was again active.

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* Loc. cit.

† Beiträge zur Kenntniss des Pancreas, Pflüger's Archiv, vol. x, p. 578.

‡ Loc. cit.

§ Chittenden and Cummins, Amer. Chem. Jour., vol. vii, p. 46–47. Also Trans. Conn. Acad., vol. vii.

‖ Jahresbericht für Thierchemie, 1883, p. 281.

Our results accord in the main with Heidenhain's; acceleration, followed by retardation of proteolytic action as seen from the following figures.

NaCl.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·2993 gram.	70·07 per cent.	100·0
0 05 per cent.	0·2635	73·65	105·1
0·2	0·3001	69·99	99·8
0·5	0·3372	66·28	94·5
1·0	0·3923	60·77	86·7
2·0	0·4352	56·48	80·6
3·0	0·4361	56·39	80·4
5·0	0·4740	52·60	75·0

Potassium bromide and potassium iodide.

The action of these two salts is wholly an accelerating one; more pronounced, however, in the case of the bromide than in the iodide. Following are the results of the experiments:

KBr.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3881 gram.	61·19 per cent.	100·0
0·05 per cent.	0·3773	62·20	101·6
0·5	0·3681	63·19	103·2
2·0	0·3795	62·05	101 4
5·0	0·3278	67·22	109·8
KI.			
0	0·3881 gram.	61·19 per cent.	100·0
0·05 per cent.	0·3575	64·25	105·0
0·5	0·3915	60·85	99 4
3·0	0·3897	61·03	99·7

Influence of gases on the proteolytic action of trypsin.

Podolinski* in Heidenhain's laboratory, has shown that oxygen gas has the power of converting the zymogen of pancreas into the active ferment and that carbonic acid is without such power; moreover, that carbonic acid added to a sodium carbonate solution of pancreatin (trypsin) retards the proteolytic action of the ferment, because the normal carbonate is thus changed into acid carbonate which does not favor the action of the ferment so well as the simple salt. Hydrogen was found to be without such action. Podolinski further found that while oxygen favored the conversion of zymogen into the ferment, it did not influence the proteolytic action the ferment. Hydrogen was also found to be without marked action on the ferment, but carbonic acid retarded decidedly the proteolytic action of the ferment in an alkaline solution.

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* Pflüger's Archiv, vol. xiii, p. 426.

We have tried the influence of three gases, such as the ferment would naturally be brought in contact with in the intestinal canal, and find that they exert a marked influence on the activity of the ferment. We employed, as in the preceding experiments, a neutral solution of trypsin, and kept the solutions saturated with the gas during the experiment, by passing a constant current through the fluid contained in a small flask. As a control, air was passed through one digestive mixture, which served to keep the powdered fibrin in an equal state of agitation and thus make accurate comparison possible.

Following are the results:

	Undigested residue	Fibrin digested.	Relative proteolytic action
Air	0·4218 gram.	57·82 per cent	100·0
Hydrogen (H)	0·3670	63·30	109·1
Carbonic acid (CO_2)	0·5665	43·35	74·9
Hydrogen sulphide (H_2S)	0·4884	51·16	88·5

From this it is seen that hydrogen gas causes a slight acceleration in the proteolytic action of the ferment, while carbonic acid causes marked retardation, which fact agrees with Podolinski's results, and shows moreover, that the action of the gas is the same in neutral and alkaline solutions. Hydrogen sulphide also causes retardation, although not so marked as carbonic acid.

Influence of alkaloid salts on the proteolytic action of trypsin.

So far as we know, no previous experiments have been tried on this subject. Our results show, so far as our experiments extend, a greater susceptibility on the part of trypsin to the action of alkaloids than the amylolytic ferment of saliva. Moreover, with trypsin, the alkaloids in only one case cause acceleration of proteolytic action. Compared with pepsin-hydrochloric acid, however, we find that neutral solutions of trypsin are not so readily affected as the former ferment, except by one alkaloid, viz: narcotine, where retarding action is very pronounced. All of the alkaloids experimented with were in the form of pure sulphates.

Morphine sulphate.

This salt exerts but little retarding action. The results are as follows:

$(Mo)_2 . H_2SO_4+5H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3875 gram.	61·25 per cent.	100·0
0·05 per cent.	0·3952	60·48	98.7
0·5	0·4105	58·95	96·2
2·0	0·4542	51·58	89·1

Atropine sulphate.

This alkaloid is very similar to morphine in its action.

$(At)_2 . H_2SO_4$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3875 gram.	61·25 per cent.	100·0
0·05 per cent.	0·3909	60·91	99·4
0·5	0·4078	59·22	96·6
2·0	0·4619	53·81	87·8

Stolnikow* has stated that a very small amount of atropine sulphate is without influence on the ferment power of a glycerine extract of pancreas, but that large amounts of the alkaloid salt, considerably diminish the proteolytic action of the ferment.

Strychnine sulphate and Brucine sulphate.

These two alkaloids produce more marked effects on the ferment, than the preceding. Of the two, as the results show, strychnine is more powerful in preventing proteolytic action.

Alkaloid salt.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4511 gram.	54·89 per cent.	100·0
$(Sr)_2 . H_2SO_4+6H_2O$.			
0·05 per cent.	0·4760	52·40	95·4
0·5	0·5792	42·08	76·6
2·0	0·6882	31·18	56·8
$(Br)_2 . H_2SO_4+H_2O$.			
0·05	0·4915	50·85	92·6
0·5	0·5480	45·20	82·3
2·0	0·5452	45·48	82·8

Narcotine sulphate.

$(Na)_2 . H_2SO_4+H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4511 gram.	54·89 per cent.	100·0
0·05 per cent.	0·5205	47·95	87·3
0·5	0·9943	0·57	1·0
2·0	1·0	0	0

This alkaloid causes a complete stopping of proteolytic action; nearly so, even when present to the extent of only 0·5 per cent.

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* Virchow's Archiv, vol. xc, p. 435.

Quinine sulphate, Cinchonine sulphate and Cinchonidine sulphate.

The following table of results shows the action of these three salts:

Alkaloid salt.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4679 gram.	53·21 per cent.	100·0
$(Q)_2 . H_2SO_4 + 7H_2O$.			
0·05 per cent.	0·4811	51·89	97·5
0·5	0·6310	36·90	69·3
2·0	0·7600	24·00	45·1
$(Ci)_2 . H_2SO_4 + 2H_2O$.			
0·05	0·4868	51·32	96·4
0·5	0·6409	35·91	67·4
2·0	0·8327	16·73	31·4
$(Cidine)_2 . H_2SO_4 + 3H_2O$.			
0·05	0·4467	55·33	104·0
0·5	0·5977	40·23	75·6
2·0	0·7707	22·93	43·0

Cinchonidine, in the smallest percentage, causes a slight acceleration in proteolytic action; retardation is also less marked with 0·5 per cent. than in the case of the other two alkaloids. Otherwise the results are much alike.

On the opposite page is a table showing relative action of the various salts on the proteolytic power of the ferment, compared with the action of the controls expressed as 100.

O. Nasse,* by a study of the influence of various salts (inorganic) on fermentation, particularly their influence on the amylolytic action of the salivary ferment, pancreatic ferment, and the invert ferment of yeast, came to the conclusion that there is a manifest and important dependence on the part of the ferments in question, in their action, on the presence of salt molecules, and moreover, that this dependence is specific for each individual ferment; that the quantity, as well as the quality of a salt, exercises a specific influence upon each ferment.

Our own results, with still different ferments, and with a larger number of substances, both related and more varied in character, all testify to the truth of this statement, viz: that the unorganized ferments are much influenced by the presence of salts, and moreover, that there is no distinct relationship among the ferments in question in their behavior towards the various salts experimented with, as a study of the three tables of comparisons show. Thus one and the same salt may affect two ferments in quite a different manner, as seen

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* Pflüger's Archiv, vol. xi, p. 157.

Table showing relative proteolytic action.

	0·0005 p. c.	0·001 p. c.	0·002 p. c.	0·003 p. c.	0·005 p. c.	0·010 p. c.	0·025 p. c.	0·05 p. c.	0·1 p. c.	0·2 p. c.	0·3 p. c.	0·5 p. c.	1·0 p. c.	1·5 p. c.	2·0 p. c.	3·0 p. c.	5·0 p. c.
$HgCl_2$	----	----	100·5	101·6	98·7	----	89·4	----	40·8	----	----	----	----	----	----	----	----
$HgBr_2$	----	----	----	----	105·8	----	97·4	----	80·8	65·2	----	----	----	----	----	----	----
HgI_2	----	----	----	----	98·6	----	92·8	----	75·6	64·6	----	----	----	----	----	----	----
$Hg(CN)_2$	----	----	----	----	92·8	----	92·6	92·1	91·2	----	93·3	96·7	----	95·9	----	----	----
$CuSO_4+5H_2O$	----	----	----	----	100·1	----	94·2	83·4	34·3	----	----	----	----	----	----	----	----
$Pb(C_2H_3O_2)_2+3H_2O$	----	----	----	----	98·2	----	84·7	79·8	66·0	----	----	0	----	----	----	----	----
$SnCl_2$	89·8	----	----	----	75·5	----	0	----	----	----	----	----	----	----	----	----	----
As_2O_3	----	99·4	----	----	101·5	----	97·9	97·6	----	----	----	----	----	----	----	----	----
H_3AsO_4	----	----	----	----	100·6	----	94·6	87·4	67 7	----	----	----	----	----	----	----	----
$(NH_4)_3AsO_4$	----	----	----	----	----	----	----	99·6	102·8	----	----	93·7	----	----	----	----	----
$K(SbO)C_4H_4O_6$	----	----	----	----	----	----	----	----	----	97·8	----	97·4	95·3	76·2	----	----	----
Fe_2Cl_6	----	----	----	----	95·4	----	78·5	53·1	0	----	----	----	----	----	----	----	----
$FeSO_4+7H_2O$	----	----	----	----	92·0	----	----	63·7	47·6	----	----	46·9	39·2	36·1	----	----	----
$MnCl_2$	----	----	----	----	100·3	----	----	101·6	94·4	----	----	66.7	----	----	----	----	----
$ZnSO_4+7H_2O$	----	----	----	----	97·2	----	84·5	64·4	34·2	----	----	----	----	----	----	----	----
$BaCl_2+2H_2O$	----	----	----	----	----	----	----	103 1	----	----	----	97 2	----	----	----	79·7	----
$MgSO_4+H_2O$	----	----	----	----	----	----	----	99·3	----	----	----	95·1	----	----	----	81·3	----
$K_2Mn_2O_8$	----	99·8	----	99·1	----	95·0	----	----	87·1	----	----	35·2	0	----	----	----	----
$K_2Cr_2O_7$	----	----	----	----	----	----	----	99·1	----	95·5	----	88·1	74·3	61·0	----	----	----
KCN	----	----	----	----	98·3	----	97·6	99 8	100·9	----	125·2	126·7	115·4	105·6	----	----	----
$K_4Fe(CN)_6+3H_2O$	----	----	----	----	95·4	----	----	----	----	----	----	96·4	----	----	89·8	----	----
$K_6Fe_2(CN)_{12}$	----	----	----	----	96·8	----	----	95·3	----	----	----	----	----	----	87 5	----	----
$Na_2B_4O_7+10H_2O$	----	----	----	----	----	----	----	106·5	----	114·5	----	130·3	138·3	----	141·7	131·2	116 5
$Na_2SO_4+10H_2O$	----	----	----	----	----	----	----	98·3	----	----	----	99·0	----	----	93·1	----	86·7
$KClO_3$	----	----	----	----	----	----	----	94·8	----	----	----	98·7	----	----	93·0	----	----
KNO_3	----	----	----	----	----	----	----	99·3	----	----	----	98·5	----	----	94·1	----	87·8
KCl	----	----	----	----	----	----	----	98·7	----	----	----	99 8	----	----	89·4	----	83·2
NaCl	----	----	----	----	----	----	----	105·1	----	99·8	----	94·5	86·7	----	80·6	80·4	75 0
KBr	----	----	----	----	----	----	----	101·6	----	----	----	103·2	----	----	101·4	----	109·8
KI	----	----	----	----	----	----	----	105·0	----	----	----	99·4	----	----	----	99·7	----
$(Mo)_2 . H_2SO_4+5H_2O$	----	----	----	----	----	----	----	98·7	----	----	----	96·2	----	----	89·1	----	----
$(At)_2 . H_2SO_4$	----	----	----	----	----	----	----	99·4	----	----	----	96·6	----	----	87 8	----	----
$(Na)_2 . H_2SO_4+H_2O$	----	----	----	----	----	----	----	87·3	----	----	----	1·0	----	----	0	----	----
$(Q)_2 . H_2SO_4+7H_2O$	----	----	----	----	----	----	----	97·5	----	----	----	69·3	----	----	45·1	----	----
$(Ci)_2 . H_2SO_4+2H_2O$	----	----	----	----	----	----	----	96·4	----	----	----	67·4	----	----	31·4	----	----
$(Cidine)_2 . H_2SO_4+3H_2O$	----	----	----	----	----	----	----	104·0	----	----	----	75·6	----	----	43·0	----	----
$(Sr)_2 . H_2SO_4+6H_2O$	----	----	----	----	----	----	----	95·4	----	----	----	76·6	----	----	56·8	----	----
$(Br)_2 . H_2SO_4+H_2O$	----	----	----	----	----	----	----	92·6	----	----	----	82·3	----	----	82·8	----	----

in the action of sodium tetraborate on the salivary ferment and on trypsin.

It is moreover, evident, from a comparison of the results obtained with the three ferments, that under the conditions of dilution, etc., with which our experiments were tried, the salivary ferment is the most sensitive to the action of the various salts, while of the other two, trypsin is as a rule most readily affected. Still, it is hardly possible to draw a direct comparison between the two proteolytic ferments, since they act under such different conditions; the fact that pepsin acts only in an acid medium and that both the strength and nature of the acid affect the activity of the ferment, introduces an additional factor which makes direct comparison in the case of the two ferments impossible. The possible reason for the slight acceleration of proteolytic action produced by several neutral salts, in the case of pepsin-hydrochloric acid, has been already referred to; but why neutral salts, which in large percentages show retarding action, should in smaller percentages added to a *neutral* solution of the ferment (ptyalin or trypsin) produce acceleration, can only be conjectured. We might assume a simple *stimulation* of ferment action by mere contact. That it may become very pronounced, is evident from the action of borax, potassium cyanide and potassium bromide in the case of trypsin and mercuric cyanide, ammonium arsenate, ferrous sulphate, potassium chlorate, sodium chloride, tartar emetic, and alkaloid salts in the case of the salivary ferment.

As to the manner in which the various salts produce retardation of ferment action, it would appear as if many of the results obtained, could be accounted for only by assuming, in addition to the views already offered, a direct influence in many cases, either destructive or hindering, on the ferment itself.

Those substances, which are particularly injurious to animal cells show in many cases no retarding action whatever, on the unformed ferments; this is particularly noticeable in the case of the arsenic compounds, which affect the ferments only as the solutions become acid. On the other hand, certain of the metallic salts are alike injurious to both and doubtless for the same reason, viz: on account of their power of combining with albuminous matter, which fact applies with equal force to the vegetable organisms (organized ferments). Nearly all germicides act injuriously on the unformed ferments. Many salts, however, well known as antiseptics, are without injurious action, except when present in large quantity; notably borax in the case of trypsin or boracic acid in the case of pepsin-hydrochloric acid.

VIII.—INFLUENCE OF TEMPERATURE ON THE RELATIVE AMYLOLYTIC ACTION OF SALIVA AND THE DIASTASE OF MALT. BY R. H. CHITTENDEN AND W. E. MARTIN, PH.B.

IT has long been known that the ferment of saliva and the diastase of malt, differ from each other in the temperature best adapted to their amylolytic action. Paschutin* states that saliva ten times diluted, converts starch into dextrin and sugar most rapidly at 38° to 41° C., while the strongest action of the malt ferment occurs at 70° C.; rising slowly from 50° C. up to this temperature. According to Kühne,† the amylolytic action of salivary ptyalin is most energetic at 35° C., while the action of malt diastase is most rapid at 66° C.; ptyalin being destroyed at 60° C. Kjeldahl‡ states that the amylolytic power of diastase rapidly increases with increase of temperature up to +50° C. By 54° C., ferment action is more vigorous than at 50° C., while maximum action lies between +54° C. and +63° C. Above +63° C. amylolytic action rapidly diminishes with increase in temperature.

Exposing diastase for a long time to a temperature below 63° C. does not, according to Kjeldahl, weaken perceptibly the action of the ferment. Higher temperatures, however, cause a diminution in amylolytic power proportional to the length of time the ferment is heated; thus Kjeldahl states that long continued warming at +66° C. produces the same effect upon diastase as heating for a shorter time at +70° C. For the ptyalin of saliva, Kjeldahl finds the temperature most favorable for amylolytic action to be about +46° C. From this temperature, amylolytic action diminishes on both sides, although somewhat more rapidly by increase in temperature.

O'Sullivan§ and Brown and Heron‖ have also studied the influence of temperature on the amylolytic action of malt extract, more however with a view to ascertaining the relative proportion of products (maltose and dextrin) formed and the nature of the change involved, than any comparison of the effect of temperature on the energy of the ferment.

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* Quoted by Hoppe-Seyler, Physiologische Chemie. p. 187.

† Lehrbuch der Physiologischen Chemie, p. 20–21.

‡ Abstract in Jahresbericht für Thierchemie, 1879, p. 381–383.

§ On the action of malt-extract on starch, Journal Chem. Soc., 1876, ii, p. 125.

‖ Beiträge zur Geschichte der Stärke und der Verwandlungen derselben. Liebig's Annalen der Chemie, vol. cxcix, p. 213. Also Journal Chem. Soc.

Hüppe* likewise, has made a study of the effect of high temperatures on the two ferments, in manner similar to the experiments of Bull, Hüfner and Salkowski, not, however, to ascertain the effects of definite temperatures on ferment action, but rather to ascertain the extent to which the dry ferments can be heated without destroying their peculiar properties.

It has been our purpose to obtain by quantitative methods, definite expressions of the influence of temperature on the relative amylolytic action of the two ferments.

Our method of determining amylolytic action, is based upon the gravimetric determination of the cupric oxide-reducing power of the solution, resulting from the action of the ferment upon starch paste, according to the method of Allihn.† This gives very concise and definite results of admitted accuracy. The cupric oxide-reducing power of a solution, resulting from the amylolytic action of these two ferments, must necessarily express the degree of intensity of ferment action, since the more energetic the action, the larger the amount of sugar (maltose and dextrose) formed, with higher reducing power; while the weaker the action, the larger the amount of dextrins with lower reducing power.

The amylaceous material employed in the experiments, was purified corn starch. In each experiment 1 gram of the starch was made into a paste with 50 c. c. of boiling water, then 40 c. c. more water were added, and lastly, when everything was in readiness, 10 c. c. of the ferment solution, either saliva or malt extract; thus making a volume of 100 c. c. containing 1 per cent. of starch. In every case, the ferment was allowed to act upon the starch at the desired temperature for exactly thirty minutes, when further ferment action was at once stopped by the addition of a definite quantity of dilute acid. The ferment being thus destroyed, the solution was neutralized by adding an amount of sodium hydroxide equivalent to the acid, after which the solution was concentrated, then made up to exactly 100 c. c., and in 25 c. c., or one-fourth of the filtered fluid, the reducing bodies were determined. From the weight of metallic copper so obtained, the reducing bodies are, for the sake of comparison, calculated as dextrose, from which in turn is calculated the percentage of starch converted. Naturally the amount of starch digested, is larger than the figures indicate, since the reducing power of maltose and the dextrins is much smaller than that of dextrose, but the above method of calculation is most convenient and for comparison quite sufficient.

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* Jahresbericht fur Thierchemie, 1881, p. 446. Ueber das Verhalten ungeformter Fermente gegen hohe Temperaturen.

† Zeitschrift für Analytische Chemie, xxii, p. 448.

The saliva employed in the experiments was filtered, human mixed saliva, carefully neutralized and then diluted; 20 c. c. saliva to 80 c. c. water. As 10 c. c. of this fluid were used in each experiment, 1 gram of starch was exposed to the action of 2 c. c. of normal saliva in a dilution of 1:50.

The malt extract was prepared from coarsely ground, malted barley by extracting 10 grams with 200 c. c. water at 40° C. for 3–4 hours; then filtering, neutralizing and diluting to 500 c. c., a few drops of thymol being added to prevent acid fermentation. The amylolytic power of 10 c. c. of this diluted extract was a little more than that of a similar quantity of the dilute saliva. Each series of experiments, however, was made with different extracts, each of which showed considerable variation in amylolytic power.

A. *Amylolytic action at definite temperatures.*

In all of these experiments, the 90 c. c. of fluid containing the starch paste was brought to the desired temperature by immersion in a large water-bath carefully regulated, the thermometer being immersed in the vessel containing the starch paste. In a similar manner, the ferment solution was quickly brought to the same temperature, care being taken in the latter case, that the fluid did not go beyond the requisite point. When the temperature became constant, 10 c. c. of the ferment solution were added and the action continued for thirty minutes.

The results are clearly expressed in the following series of tables:

SERIES I.[*]—SALIVA.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
10° C.	0·0935 gram.	0·1904 gram.	17·19 per cent.
20	0·1227	0·2496	22 46
40	0·1410	0·2872	23 83
50	0·1003	0·2040	18·35

SERIES II.—SALIVA.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
20° C.	0·0891 gram.	0·1816 gram.	16·34 per cent.
30	0·0945	0·1924	17·31
40	0·1203	0·2448	22·03
50	0·1419	0·2888	25 99
55	0·1129	0·2296	20·66
60	0·0451	0·0936	8·42
65	0·0125	0·0282	2·53

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[*] It is of course understood, that the results in any one series are obtained with the same ferment solution.

Series III.—Saliva.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
20° C.	0·1177 gram.	0·2396 gram.	21·56 per cent.
30	0·1273	0·2592	23·32
40	0·1285	0·2616	23·54
45	0·1174	0·2392	21·52
50	0·1131	0·2300	20·70
55	0·1029	0·2092	18·82
60	0·0883	0 1800	16·16*
65	0·0354	0·0748	6 73
70	0·0017		

Series IV.—Saliva

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
25° C.	0·0748 gram.	0·1528 gram.	13·75 per cent.
30	0·0897	0·1828	16 45
40	0·1070	0·2180	19·62
45	0·0990	0·2016	18.18
50	0·0806	0·1644	14·79
55	0·0715	0·1460	13.14
60	0 0278	0·0596	5·36
65	0·0142	0·0328	2·95

Series V.—Saliva.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
40° C.	0·1062 gram.	0·2164 gram.	19 47 per cent.
2	0·0611	0·1252	11·33

Series VI.—Malt Extract.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
30° C.	0·1374 gram.	0·2800 gram.	25.20 per cent.
40	0·1520	0·3100	27·90
50	0·1606	0·3280	29·52
60	0·1408	0·2864	25·77
70	0·0544	0·1124	10·11
80	0·0127	0·0296	2·66

Series VII.—Malt Extract.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
35° C.	0·1573 gram.	0·3208 gram.	28·87 per cent.
45	0·1552	0·3168	28·51
50	0·1575	0·3212	28·90
55	0·1617	0·3300	29.70
65	0·0585	0·1200	10·80
75	0·0366	0·0768	6·91

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* See remarks further on, in regard to this high result.

SERIES VIII.—MALT EXTRACT.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
30° C.	0·1301 gram.	0·2648 gram.	23·83 per cent.
40	0·1471	0·2996	26·96
45	0·1542	0 3148	28·33
50	0·1488	0·3036	27·31
55	0·1320	0·2688	24·19
60	0·0691	0·1412	12·70
65	0·0654	0·1340	12·06

SERIES IX.—MALT EXTRACT.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
30° C.	0·1283 gram.	0·2612 gram.	23·50 per cent.
35	0·1435	0·2924	26·31
40	0·1507	0·3072	27·64
45	0·1562	0·3188	28·69
48½	0·1573	0·3208	28·87
50	0·1588	0·3244	29·19
55	0·1445	0·2944	26·45
60	0·0742	0·1516	13·64
65	0·0561	0·1152	10·36

SERIES X.—MALT EXTRACT.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
40° C.	0·1419 gram.	0·3042 gram.	27·36 per cent.
2	0·0299	0·0636	5·72

By a study of these results, it is evident that in the case of the salivary ferment, variations in amylolytic action are not very great between the temperatures of 20° and 50°, or even 55° C. With the temperatures experimented with, however, amylolytic action appears to reach its maximum, in the case of saliva, at 40°; although in one single instance, for some unaccountable reason it appeared to be greater at 50° C. With the diastase of malt on the other hand, amylolytic action reaches its maximum at 50° C., although in one instance it appeared somewhat greater at 55° C.; great variations, however, are not to be observed between the temperatures of 30° and 55° C. Brown and Heron,* working with extract of malt and pure potato starch at different temperatures, obtained results by determination of both specific rotary power and cupric oxide-reducing power, which point to the same conclusions as those obtained by us. Thus at 40° C., the malt extract having been previously heated at the same temperature for 20 minutes, these investigators found at the end of 30 minutes, as a result of the action of the malt extract on the starch $(\alpha)_j = 163{\cdot}3°$, while at 50° C. under the same conditions $(\alpha)_j = 162{\cdot}7°$ and at 60° C. $(\alpha)_j = 164{\cdot}1°$, or in a second experiment, $(\alpha)_j = 163{\cdot}7°$. These results show at 50° C. the formation of a little more maltose than at 40°, although the difference is very slight; while at 60° C. the amount of maltose formed, is less even than at 40° C. Evidently then, the maximum amylolytic action of diastase of malt takes place at temperatures far below 60° C.; even below 55° C.

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* Liebig's Annalen der Chemie, vol. cxcix, p. 221. Also Journal Chem. Soc., 1879.

At very low temperatures, there is a corresponding difference in the action of the two ferments, as is apparent from the results obtained at 2° C.; the ferment of saliva being comparatively far more active at this temperature than the ferment of malt.

Hence it is apparent throughout, that diastase requires a higher temperature than the salivary ferment, in order to act with equal vigor; at the same time it is evident that at the body temperature, say 40° C., the difference in action between the two ferments is not very great. At 80° C. the diastase of malt still acts upon starch, although only slightly; the salivary ferment, however, under the conditions of our experiments, does not act at all at 70° C. and only slightly at 65° C. With these higher temperatures, it makes considerable difference in the ultimate result, whether the ferment solution is quickly brought to the desired temperature or not, and whether it remains long at the temperature in question, before being added to the starch solution. Thus, in the action of saliva at 60° C., if the ferment be warmed quickly to *nearly* 60° C., say 59° C., and then added to the starch paste at 61° C., as was done in the case of Series III, amylolytic action is considerably greater than when the saliva is actually brought to 60° and kept there for a moment or so to be sure of its constancy. Some variation in the length of time, required to bring the ferment solution to the desired temperature, was unavoidable, and doubtless, slight variations in the results at higher temperatures, occur from this cause. It was not, however, our purpose at this time, to heat the ferment in order to induce a change in its character, but simply to prevent any alteration in the temperature of the starch mixture on addition of the ferment, so that the action of the ferment on the starch might take place at a constant temperature.

The effects, on amylolytic action, of exposing the ferment of saliva to different temperatures for varying lengths of time.

Brown and Heron* state that a malt extract, warmed quickly to 66° C. and then added at once to starch paste at the same temperature, differs but little, in the first stages of its action, from a malt extract heated at 60° C.; if, however, the malt extract be warmed for say 10 or 15 minutes at this temperature, previous to adding it to the starch paste, its amylolytic action is very much weakened. Evidently then, under the influence of the increased temperature, a portion of the ferment is destroyed or else changes are induced, by which the action of the ferment is modified. Results of like nature were previously obtained by O'Sullivan,† with malt extract.

With saliva, we have tried the following experiments, designed originally to throw light on the comparative destructibility of the ferment.

SERIES XI.—SALIVA.

The saliva was exposed to the designated temperature for the specified time, then added to the starch paste at the *same temperature* and its amylolytic power determined.

Temperature.	Time of exposure.	Wt Cu in ¼.	Total amount reducing bodies.	Starch converted.
60° C.	0 min.	0·0409 gram.	0·0852 gram.	7·66 per cent.
60	15	0·0213	0·0464	4·17
60	30	0·0210	0·0460	4·14

At 60° C. therefore, the coagulating point of albumin and the temperature at which ptyalin is supposed to be destroyed, it is apparent that destruction of the ferment is not complete even by 30 minutes exposure to this temperature. The peculiarity of the results, moreover, make it doubtful whether we have to do with destruction at all. If the reduced amylolytic action is due to simple destruction of the ferment, we should expect less ferment action after 30 minutes exposure than after 15 minutes; as it is, the action in the two cases is the same. A certain time, however, is required to produce the change in the character of the ferment. Similar results are shown in the following series of experiments, conducted in the same manner as the preceding, only at different temperatures.

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* Loc. cit., p. 227.

† Loc cit, p. 143.

SERIES XII.—SALIVA.

Temperature.	Time of exposure.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
50° C.	15 min.	0·0773 gram.	0·1576 gram.	14·18 per cent.
50	60	0·0765	0·1560	14·04
55	30	0·0474	0·0984	8·85
55	60	0·0414	0·0864	7·21

Comparing these results with those obtained at like temperatures in Series I.–III., it is seen that a few minutes exposure at the designated temperature, lowers materially the amylolytic power of the solution, while doubling the time of exposure does not materially affect the result; a fact which is not consistent with the view that diminution in amylolytic power, under these conditions, is due to gradual destruction of the ferment.

The following series of experiments, also with saliva, throw additional light on the action of high temperatures on this ferment. In these two series, the saliva was exposed to the designated temperature for the specified time, then *cooled to* 40° C. and added to the starch paste *at a like temperature.*

SERIES XIII.—SALIVA.

Temperature.	Time of exposure.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
40° C.	0 min.	0·1081 gram.	0·2200 gram.	19·80 per cent.
50	30	0·1026	0·2088	18·79
55	30	0·0986	0·2008	18·07
60	30	0·0279	0·0596	5·36

SERIES XIV.—SALIVA.

Temperature.	Time of exposure.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
40° C.	0 min.	0·1062 gram.	0·2164 gram.	19·47 per cent.
55	180	0·0798	0·1628	14·65

It would appear from these results, that by exposure of the saliva to 50° or 55° C., in the latter case for even 3 hours, and then cooling to 40° C. and testing the amylolytic power of the ferment at that temperature, less diminution of ferment action is to be observed. This speaks still more strongly against destructive action, by simple coagulation, and at the same time suggests that not only does exposure to say 55° C. affect the character of the ferment, but also that the action of the ferment so treated, is in a given time different at that same temperature from what it is at 40° C., or the temperature of maximum action. This latter point, however, which is contrary to the law laid down by Brown and Heron* for malt extracts at temperatures above 50° C. we reserve for further investigation. Owing to the great difficulty in rendering saliva perfectly neutral, it is possible that the observed low result at 55° C. in Series XII. may be due to the presence of a trace of either acid or alkaline carbonate.

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* Liebig's Annalen der Chemie, vol. cxcix, p. 221. Also, Journal Chem. Soc., 1879.

Finally, it is to be observed that the majority of the results obtained, indicate that the influence of different temperatures, on the amylolytic action of the salivary ferment, is due rather to change in the character of the ferment, than to the direct influence of the various degrees of heat upon the cleavage of the starch molecule; similar in character to that indicated by the work of O'Sullivan, and also of Brown and Heron in the case of malt diastase.

IX.—INFLUENCE OF BILE, BILE SALTS AND BILE ACIDS ON AMYLOLYTIC AND PROTEOLYTIC ACTION.* BY R. H. CHITTENDEN AND GEO. W. CUMMINS, PH.B.

THE influence of bile and bile acids on the digestive processes of the intestinal canal has long been considered an important one, still few experiments have been made to determine the exact influence of these substances by themselves on ferment action. The form in which the main constituents of the bile exist in the intestinal canal depends naturally upon the reaction of the contents of the intestines. If these have an acid reaction, bile acids must be present; if alkaline, salts of these acids; and it is fair to presume that under these two conditions the presence of bile may be productive of different effects on ferment action. Recorded observations tend to show that ordinarily the contents of the intestines possess a distinct acid reaction; thus Schmidt-Mülheim† has found that in dogs fed on albuminous matter, the contents of the small intestines are invariably acid, although the mucous membrane sometimes possesses an alkaline reaction. It is evident that in such cases the alkali of the bile must have combined with the acid of the chyme, which would be followed by liberation of the bile acids and partial precipitation of the same in combination with the proteid matters of the chyme. Moreover, the recorded observations of Schmidt-Mulheim tend to show that this acid condition of the contents of the intestines persists throughout the entire length of the intestinal canal. Uffelmann‡ has likewise found, in corroboration of the above, that the faeces of infants naturally nourished possess a weak acid reaction, while, on the other hand, Nothnagel,§ as a result of 800 observations, finds that human excrement, in the case of adults, varies decidedly in its reaction, being generally alkaline, more rarely acid or neutral. It is hardly proper, therefore, to conclude that it is only necessary to study the influence of the bile acids in their free condition on ferment action, since in the passage of the ferments through the intestinal canal there are times, doubtless, when the reaction of the mass is more or less alkaline, especially in the small intestines, for some distance beyond the opening of the bile and pancreatic ducts. In either case it is an interesting point to ascertain whether the bile salts have an action at all analogous in kind or extent to that of the free acids.

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* Also published in the American Chemical Journal, vol VII, p. 36

† Archiv fur Physiologie, DuBois Reymond, 1879, p. 56.

‡ Jahresbericht fur Thierchemie, 1881, p. 305.

§ Jahresbericht fur Thierchemie, 1881, p. 309.

Many observations* are recorded concerning the duodenal precipitate formed in the duodenum by the action of bile on the acid-reacting chyme. The precipitate itself has generally been supposed to consist of a mixture of syntonin, peptone and bile acids, but recent experiments of Maly and Emich† with pure bile acids tend to show that only the non-peptonised albuminous bodies are precipitated, viz: coagulable albumin and syntonin, and these only by taurocholic acid, while peptone and "propeptone" remain in solution. This fact lends favor to the view advanced by Hammarsten, that the object of the precipitation of albuminous matter on the walls of the intestines is to prevent its too rapid passage through the intestinal canal, thus giving ample opportunity for the action of the pancreatic juice.

The addition of taurocholic acid to a solution of peptone, Maly and Emich find, is followed by the formation of a distinct opalescence or fine dust-like precipitate, slowly changing to fine droplets. This precipitate, however, which is doubtless the same as observed by Hammarsten and Brücke on the addition of bile to portions of a digestive mixture, does not contain according to Maly and Emich, any peptone, but consists of taurocholic acid, possibly in a modified form.

Both of these precipitations, however, would tend to mechanically throw down, to a greater or less extent, any ferment present, and thus diminish ferment action; but, as Maly points out, the main reason for a diminished action, in the case of pepsin, is to be sought for, not in a precipitation of the ferment, but in the formation of a compound of albumin with the bile acid, not digestible by pepsin-hydrochloric acid. But since this precipitation, as a normal reaction in the animal body, must take place in the intestinal canal, it is equally important to ascertain the extent of its digestibility in pancreatic juice, or, in other words, to ascertain the exact influence of bile and its several constituents on the proteolytic action of trypsin as well as on the action of pepsin and on amylolytic action. The only data bearing on these points are the recent experiments of Maly and Emich, who have found that 0·2 per cent. taurocholic acid hinders the digestive action of pepsin-hydrochloric acid, while 1 per cent. of glycocholic acid is without influence. The same investigators likewise state that 0·1 per cent. taurocholic or glycocholic acid stops the amylolytic action of the pancreas ferment, and that 0·2 per cent. taurocholic acid or 1 per cent. glycocholic acid will completely stop the amylolytic action of the salivary ferment.

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* See Maly in Hermann's Handbuch der Physiologie, vol. v, p. 180.

† Monatshefte fur Chemie, vol. iv, p. 89.

Our experiments on this subject were commenced before the above results were published, and we have continued them, since we wished to ascertain likewise the influence of the bile salts, and also the effects of both salts and acids, as well as the bile itself, on the proteolytic ferment of the pancreas. The results of Maly and Emich, moreover, not being quantitative, do not express the relative effects of the various percentages of bile acids used, but simply the percentage of acid necessary to stop ferment action under the conditions described by them.

1.—*Influence on Amylolytic Action.*

As amylolytic ferment, we have employed filtered human mixed saliva made neutral and then diluted to a known volume. In studying the influence of the various percentages of bile salts and acids on the action of the ferment, we have used a digestive mixture (50 or 100 cc.) containing 1 per cent. of starch previously boiled with water, and 2 per cent. of saliva, together with the given percentages of bile salts or acids. The extent of diastatic or amylolytic action under the varying conditions was determined in each case by estimating the amount of reducing substances, maltose and dextrose, formed during 30 minutes warming at 40° C. Further diastatic action was at once stopped by boiling the digestive mixtures, after which they were diluted to a known volume, and the reducing substances determined in a given portion of the diluted fluid by Allihn's gravimetric method.* The reducing substances are in each instance calculated as dextrose, and the diastatic action is expressed in the percentage of starch converted into sugar.

We first tried the influence of crystallized ox bile, since bile itself contains a small amount of a diastatic ferment. A 1 per cent. solution of nicely crystallized ox bile was made, with which the following results were obtained:

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* Zeitschrift fur analytische Chemie, xxii, 448.

Crystallized bile.	Wt. Cu in ⅛.*	Total amount reducing bodies.	Starch converted.
0 per cent.	0·0643 gram.	0·2636 gram.	23·72 per cent.
0·01	0·0630	0·2584	23·25
0·02	0·0686	0·2804	25·23
0·03	0·0693	0·2836	25·52
0·05	0·0656	0·2688	24·19
0·10	0·0734	0·3000	27·00
0·20	0·0665	0·2724	24·51
0·35	0·0447	0·1860	16·74

Here it is plain that a mixture of sodium glycocholate and taurocholate, in such proportion as they are contained in crystallized ox bile, exerts no appreciable retarding influence on amylolytic action until present to the extent of 0·35 per cent. On the contrary, smaller percentages unmistakably tend to increase the diastatic action of the ferment. The solution of crystallized bile had, however, a slight acid reaction, and possibly this may have had some influence in giving the latter results. The saliva and starch were both neutral.

Experiments were next tried with sodium taurocholate alone, and also with sodium glycocholate. Following are the results:

Sodium taurocholate.	Wt. Cu in ⅛.	Total amount reducing bodies.	[illegible] converted.
0 per cent.	0·0787 gram.	0·3212 gram.	28·90 per cent.
0·3	0·0030	0·0146	1·51
0·5	0·0023	0·0112	1·00
Sodium glycocholate.			
0·5	0·0783	0·3196	28·76

It is thus plainly evident that sodium taurocholate has a very decided action on the amylolytic ferment of saliva, while the same percentage of glycocholate is entirely without effect. The retarding action of crystallized bile is thus, without a doubt, due wholly to the taurocholate. Moreover, even smaller percentages of sodium taurocholate retard amylolytic action with almost equal energy.

The following results were obtained under like conditions as the preceding, except that the 2 per cent. of saliva employed was not neutralized.

Sodium taurocholate.	Wt. Cu in ⅛.	Total amount reducing bodies.	Starch converted.
0 per cent.	0·0590 gram.	0·1212 gram.	21·81 per cent.
0·14	0·0079	0·0192	3·45
Sodium glycocholate.			
0·20	0·0758	0·1548	27·86

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* One-eighth of the entire digestive mixture.

Thus even 0·14 per cent. of sodium taurocholate under these conditions almost entirely stops amylolytic action. The smaller percentage of glycocholate, however, causes the same increased amylolytic action observed with the smaller percentages of crystallized bile.

With the bile acids the following results were obtained. The glycocholic acid used was a nicely crystallized specimen prepared from ox bile, while the taurocholic acid, prepared from the same source, was amorphous:

Per cent. bile acid.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·0694 gram.	0·1420 gram.	25·56 per cent.
0·01 taurocholic.	0·0753	0·1538	27·68
0·05	0·0783	0·1598	28·76
0·10	0·0060	0·0146	2·63
0·20	0		
0·05 glycocholic.	0·0523	0·1082	19·47
0·10	0·0095	0·0234	4·21
0·20	0·0056	0·0136	2·44
0·50	trace		
1·00	0		

It is thus seen that 0·1 per cent. taurocholic acid prevents amylolytic action almost entirely, while 0·2 per cent. does not allow the conversion of any starch into sugar. This agrees exactly with the results obtained by Maly and Emich.* These same investigators, however, found only a trace of amylolytic action in the presence of 0·05 per cent. taurocholic acid; a result which does not agree with what we have found, working, however, under somewhat different conditions.

The presence of 1·0 per cent. glycocholic acid entirely prevents the conversion of starch into sugar, while 0·5 per cent. allows only the smallest amount of diastatic activity. Maly and Emich likewise found that 1·0 per cent. of glycocholic acid stopped the diastatic action of saliva.

We have repeated the last series of experiments in part, using, however, normally alkaline saliva instead of neutralized.

Per cent. bile acid.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·0590 gram.	0·1212 gram.	21·81 per cent.
0·1 glycocholic.	0·0107	0·0258	4·64
0·2	0·0057	0·0139	2·50
0·1 taurocholic.	0·0052	0·0126	2·26

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* Loc. cit., p. 118.

These results agree exactly with the preceding, and both together plainly show that only small percentages of bile acids are required to entirely prevent the amylolytic action of saliva. Assuming that the amylolytic ferment of the pancreatic juice is similar in its nature to the ferment of saliva, it would follow from our experiments that whether the contents of the intestines are acid or alkaline, the presence, beyond a certain percentage, of taurocholic acid, either as free acid or as a taurocholate, would tend to diminish amylolytic action. Very small percentages, however, would have little, if any, retarding effect, indeed might increase amylolytic action. As to glycocholic acid, the free acid is much more powerful in its action on the amylolytic ferment than the sodium salt of the acid.

Considering these results in the light of a possible application to changes in the intestinal canal, it becomes an interesting point to ascertain whether bile itself exerts the same influence on amylolytic action as the bile salts. Moriggia and Battistini* state that while bile mixed with chyme gives a precipitate which, among other things, contains mucin, bile acids and pepsin, thus hindering gastric digestion, it does not, on being mixed with saliva, hinder its amylolytic action. This they found to be the case both with bile containing mucin and with bile from which the mucin had been removed by acidifying. We have, therefore, made the following experiments with fresh ox bile containing 7·46 per cent. of solid matter. The digestive mixtures contained as before 1 per cent. of starch, 2 per cent. of neutral saliva, and were warmed at 40° C. for 30 minutes:

Ox bile.	Wt. Cu in ⅙.	Total amount reducing bodies.	Starch converted.
0 per cent.	0·0753 gram.	0·3072 gram.	27·64 per cent.
2·0	0·0875	0·3568	32·11
5·0	0·0690	0·2824	25·41
10·0	0·0719	0·2944	26·50
20·0	0·0770	0·3144	28·30

Here in close accord with what has been found before, the presence of a small percentage of bile causes increased amylolytic action; larger percentages, however, have little, if any, effect; certainly not such an effect as would be expected from the known action of the bile salts. The bile itself possessed to a slight extent, diastatic action; 20 c. c. of the bile (20 per cent.) converting 4·53 per cent. of the starch into sugar in 30 minutes. This, however, could hardly account for the increased amylolytic action noticed above in the presence of 2 per cent. of bile. Wittich* and also Hofmann have noticed the occasional diastatic action of bile, Wittich even extracting the ferment from human bile by his glycerine method. Gianuzzi and Bufalini† have shown that the action varies considerably in bile from different animals and individuals, and without any apparent dependence upon the nature of the food. Ewald‡ states that the diastatic capacity of bile appears to be slight in all cases, and is not found in bile which has stood for some time. We have found, however, in bile from several animals considerable diastatic power; thus in one sample of fresh sheep's bile, 25 c. c. (25 per cent.) converted 24·33 per cent. of starch into sugar in 30 minutes at 40° C. We have likewise found great variation in diastatic power, varying, expressed in the percentage of starch converted into sugar under the conditions described, from 4 to 24 in the case of herbivorous animals. We have also noticed in bile from sheep and oxen the presence of a small amount of sugar, or at least a substance capable of reducing Fehling's solution. In one instance the amount was not inconsiderable; 25 grams of ox bile yielding, by Allihn's method, 0·040 gram metallic copper, equal to 0·0209 gram dextrose or 0·08 per cent. Naunyn, we believe, has already claimed the presence of sugar in bile.

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* Jahresbericht fur Thierchemie, 1876, p. 196.

While we know then that bile acids and bile salts by themselves retard very decidedly the amylolytic action of ptyalin, it would appear that the retarding influence of the latter may be, in part at least, counteracted by other substances naturally present in the bile.

2.—*Influence on the Proteolytic Action of Pepsin.*

It has long been known that bile has a retarding action on pepsin digestion, and Maly and Emich have recently shown the percentages of bile acids necessary to bring the action of pepsin to a standstill. We have, however, in addition, experimented with bile itself, and as in the case of the amylolytic ferment, have endeavored to study the influence of the bile acids quantitatively. The method employed for measuring proteolytic action is one frequently used in this laboratory, and which has invariably given satisfactory results. The only feature which calls for description is the preparation of the proteid matter to be digested. The material consists of carefully selected and thoroughly washed blood fibrin. All soluble matters are removed by successive extraction with boiling water, cold and boiling alcohol, and finally with cold and warm ether. The fibrin is thus obtained in a perfectly friable condition and can be easily ground to a coarse powder. It is then dried at 100–110° C. This material is well adapted for quantitative experiments with pepsin-hydrochloric acid; the residue remaining after a digestion can be rapidly filtered with the aid of a pump, and can be easily freed, by washing, from peptones and other soluble products of digestion.

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* Jahresbericht fur Thierchemie, 1872, p. 243.

† Jahresbericht für Thierchemie, 1876, p. 197.

‡ Lectures on digestion, Amer. ed., p. 77.

The gastric juice employed in the experiments, consisted of a hydrochloric acid solution of a glycerine extract of the mucous membrane from a pig's stomach, in the proportion of 10 grams glycerine extract to 1 litre of 0·2 per cent. hydrochloric acid. 50 or 100 c.c. of this pepsin-hydrochloric acid were employed in each experiment, to which was added 1 or 2 grams of the dried fibrin (2 per cent.), together with the given percentage of bile or bile acids.

We first tried the influence of bile itself, using fresh ox bile, slightly alkaline in reaction and containing 10·02 per cent. of solid matter.

The digestive mixtures were warmed at 40° C. for two hours, then filtered at once, and the undigested residue washed thoroughly,* and dried at 100° C. until of constant weight. Following are the results of the first series of experiments, with 2 grams of fibrin and 100 c. c. of gastric juice.

Bile in digestive mixture.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·1957 gram.	90·21 per cent.
0·25	0·1890	90·55
0·50	0·2050	89·75
1·00	0·2234	88·83
3·00	0·5453	72·73
5·00	0·7642	61·84

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* In all of the pepsin-hydrochloric acid digestions the presence of bile or bile salts naturally causes more or less of a precipitate, dependent in amount upon the percentage of bile and also upon the amount of digestive products. In washing the undigested fibrin it was of course necessary to remove this precipitate. This was accomplished by pouring over the precipitate on the filter 50 c. c. of 0·5 per cent. potassium hydroxide and then washing with water until the alkali was wholly removed.

The following experiment shows that under these conditions the alkali affects the swollen fibrin but little, if any. Two portions of fibrin of 2 grams each were warmed with 100 c. c. of 0·2 per cent. HCl for 30 minutes, then filtered and one washed with water alone, the other with water and alkali. The first gave 1·9272 grams dried residue, the other 1·9155 grams.

A second series, tried under the same conditions, but with larger percentages of bile gave the following results:

Bile in digestive mixture.	Weight of undigested residue.	Fibrin digested
0 per cent.	0·1979 gram.	90 10 per cent.
0·25	0·2456	87 72
0·50	0·1927	90·36
9·00	1·1955	40·22
13·00	1·6611	16 94
16·50	1·7812	10 94
20·00	1·9241	3 29

From these two series of experiments it is evident that the presence of bile, from 1 per cent. upward, causes diminished proteolytic action, the retarding effect being proportionate to the amount of bile present. 20 per cent. of bile stops the action, under these conditions, almost completely. It is fair to presume, therefore, that the reflux of but a small amount of bile into the stomach would be productive of a diminished proteolytic action.

These results, therefore, agree with the older statements of Brücke, Hammarsten and others, to the effect that bile added to a gastric digestion has the effect of bringing the proteolytic action to a standstill. We next tried the influence of the individual bile acids with the following results:

Taurocholic acid.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·1311 gram.	86·89 per cent.
0·025	0·1461	85·39
0·050	0·2200	78 00
0·100	0·2421	75 79
0·200	0·2668	73 32
0·500	0·3579	64·21

Here it is seen that the smallest percentage of taurocholic acid added, produces a distinct effect on proteolytic action, and in the next series of experiments still smaller percentages of acid cause an equally marked effect. In both series of experiments, the mixtures were warmed at 40° C. for 1 hour and 30 minutes.

Taurocholic acid.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·1499 gram.	85·01 per cent
0·010	0·1819	81·81
0·015	0·1900	81·00
0·020	0·2947	70·53
0·050	0·3110	68·90

Adding taurocholic acid to the digestive mixture in the form of a sodium salt has the effect of diminishing still further the action of the ferment; doubtless, due in part to the percentage of free hydrochloric acid being diminished by decomposition of the taurocholate.

Taurocholic acid.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·2059 gram.	79 41 per cent.
0·1	0·6198	38·02
0·2	0·6426	35·74
0·5	0·6475	35·25

Maly and Emich found that 0·2 per cent. taurocholic acid entirely stopped the action of pepsin; in our experiments, however, ferment action was still manifest even in the presence of 0·5 per cent. of the acid. Whether this difference in result is due to difference in the acid used, or to difference in method, we cannot say. Glycocholic acid we found to be entirely without influence on the action of pepsin, as did also Maly and Emich.

3.—*The Proteolytic Action of Trypsin in Neutral, Alkaline and Acid Solutions.*

The trypsin solution was prepared according to Kühne's method,* from dried pancreas freed from fat; the solution after neutralization always contained some sodium salicylate, sufficient to prevent putrefaction during short digestive periods. According to Kühne,† trypsin acts quite energetically, both in neutral and in salicylic acid solutions, but most energetically when the pancreatic solution contains 0·3 per cent. sodium carbonate. According to Heidenhain,‡ the action of definite percentages of sodium carbonate varies with the amount of ferment.

We have tried quantitative experiments as a preliminary to studying the influence of bile, with the following results;§ the mixtures were warmed at 40° C. for 3 hours and 40 minutes, and contained 2 per cent. of fibrin.

Reaction of the fluid.	Weight of undigested residue.	Fibrin digested.
neutral	0·2312 gram.	76.88 per cent.
0·1 per cent. Na_2CO_3	0·1570	84·30
0 2	0·0925	90·75
0·3	0·0772	92·28
0·4	0·0426	95·74
0·5	0·1038	89·62
0·1 pr. ct. salicylic acid	0·5651	43·49

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* Untersuchungen aus der physiolog. Inst. d. Universität Heidelberg, vol. i, p. 222.

† Ibid, p. 223.

‡ Pflüger's Archiv, vol. x, p. 576.

§ The pancreatic juice was prepared from 20 grams dry pancreas, and finally diluted to 1000 c. c. 50 c. c. were used in each digestion with 1 gram of pure fibrin.

With a larger percentage of fibrin and a longer period of digestion the results are somewhat different. The following were obtained with 4 per cent. of fibrin in 6 hours and 40 minutes at 40° C.:

Reaction of the fluid	Weight of undigested residue	Fibrin digested
neutral	0·3785 gram.	62·15 per cent.
0·1 per cent. Na_2CO_3	0·2581	74·19
0·2	0·1395	86·05
0·3	0·1588	84·12
0·4	0·1629	83·71
0·5	0·1318	86·82
0·1 pr. ct. salicylic acid	0·4728	52·72

An average of the two series of results plainly shows that there is but little difference in digestive action in the presence of 0·2–0·5 per cent. sodium carbonate, although in a given solution a change in the percentage of alkali is at once manifest, to a slight extent, in the amount of fibrin digested. Greatly increased percentages of alkaline carbonate materially diminish the action of the ferment, as the following series of experiments indicate; the mixtures were warmed for 2 hours at 40° C.:

Reaction of the fluid.	Weight of undigested residue.	Fibrin digested.
neutral	0·5863 gram.	41·37 per cent.
0·5 per cent. Na_2CO_3	0·1584	84·16
1·0	0·3760	62·40
2·0	0·7010	29·90
3·0	0·7892	21 08
4·0	0·8373	16·27
5·0	0·8608	13·92

The difference in action between a neutral trypsin solution and a solution containing salicylic acid is quite noticeable, at the same time it is evident that in the acid-reacting fluid the ferment simply acts more slowly, and if time be given, the action will approach more closely to that of the neutral solution. It is of course understood that the salicylic acid in the above experiments does not exist in a free state, but in combination with the proteid matter present, and doubtless in most of the experiments recorded, where trypsin has been exposed to the action of small fractions of a per cent. of acid, no free acid has been present, but only varying percentages of acid-proteids.* Kühne† has pointed out that hydrochloric acid above 0·05 per cent. is injurious to the action of trypsin, and Heidenhain‡ has shown that the addition of 0·1 per cent. hydrochloric acid to an aqueous extract of the pancreas stops its action. C. A. Ewald* however, found that while pepsin-hydrochloric acid destroyed trypsin, trypsin could digest fibrin in the presence of 0·3 per cent. hydrochloric acid. Mays† likewise found that trypsin digestion could take place in the presence of 0·3 per cent. hydrochloric acid, but only when a relatively large proportion of fibrin was present, and in corroboration of Kühne's statement, he showed that trypsin could be destroyed both by pepsin and dilute hydrochloric acid. Engesser‡ found that a pancreatic juice did not lose its tryptic power by two hours warming with a gastric juice containing 0·5 per cent. hydrochloric acid. Langley,§ on the contrary, has shown that a glycerine extract of the pancreas loses a very appreciable amount of trypsin when warmed for two and a half hours with 0·05 per cent. hydrochloric acid. Lindberger,‖ working with an alcohol precipitate from a glycerine extract of ox pancreas, in which there would naturally be present but a small amount of proteid matter in addition to the trypsin, found that in the presence of 0·1 per cent. hydrochloric acid the ferment was entirely without action, and even in the presence of 0·012 per cent. hydrochloric acid, fibrin was much more slowly dissolved than by a neutral trypsin solution. Lindberger, moreover, found that weaker acids, as acetic and lactic, had a much different effect than the stronger hydrochloric acid; thus with dilute acetic acid, digestion of the fibrin was almost as rapid as with a neutral solution of trypsin, while with small amounts of lactic acid, ferment action was even more energetic than in a neutral solution. There is, however, no guarantee that in these experiments free acid was present.¶

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* See Danilewsky. Centralbl. med. Wiss., 1880.

† Verh. Naturhist. med. Vereins zu Heidelberg, 1877, p. 193.

‡ Pflüger's Archiv, vol. x, p. 578.

We have found that *free* acids, even when present in small percentages, completely stop the proteolytic action of trypsin, and that when considerable albuminous matter is present, the action of trypsin is much hindered by the addition of acid to a neutral solution, even before the proteid matters present are saturated with acid. 0·1 per cent. *free* salicylic acid, in the presence of proteids already saturated with the acid, allows no proteolytic action whatever. Furthermore, a sufficient amount of proteid matter just saturated with hydrochloric acid, no free acid being present, will almost completely stop the action of trypsin. Proteid matter, however, only partially saturated with acid has a much smaller retarding action. This, doubtless, was the condition of the mixtures in Mays' and Engesser's experiments above referred to, for, as Mays states, the ferment could act in the presence of 0·3 per cent. hydrochloric acid only when a relatively large proportion of fibrin was present.

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* Jahresbericht für Thierchemie, 1880, p. 297.

† Untersuchungen a. d. physiolog. Inst. d. Univ. Heidelberg, vol. iii, p. 378, 1880.

‡ Jahresbericht für Thierchemie, 1880, p. 297.

§ Journal of Physiology, vol. iii, No. 3.

‖ Jahresbericht für Thierchemie, 1883, p. 281.

¶ We have seen only the abstract of Lindberger's paper, so cannot speak positively on this point.

A pancreatic juice prepared from 20 grams of dried pancreas by warming it at 40° C. with 200 c.c. 0·1 per cent. salicylic acid, etc., was finally made exactly neutral and diluted to 500 c.c.; 25 c.c. of this solution required 7·5 c.c. of a 2·0 per cent. solution of salicylic acid to completely saturate the proteids present,* the excess of free acid necessary to give the tropæolin reaction being deducted.

Three digestive mixtures were made as follows:

1. 25 c.c. of the neutral pancreatic solution + 50 c.c. water.

2. 25 c.c. of the same pancreatic solution + 7·5 c.c. 2·0 per cent. salicylic acid solution + 17·5 c.c. water. The mixture was acid to test papers, but gave no reaction with tropæolin 00. It therefore contained no free acid, but 0·3 per cent. of combined acid.

3. The same as No. 2, but 2·5 c.c. more of 2·0 per cent. salicylic acid, so that the solution contained, in addition to the acid proteids, 0·1 per cent. free salicylic acid.

One gram of fibrin was added to each of these and the mixtures warmed at 40° C. for 8 hours and 40 minutes. No. 1 digested 88·34 per cent. of the fibrin, No. 2, 13·44 per cent., while No. 3 had no action whatever.

Much smaller percentages of combined salicylic acid cause an equally diminished proteolytic action; thus, in the case of a carefully dialyzed juice where the proteid matter was much diminished, the digestive mixture, with its proteids wholly saturated, contained but 0·060 per cent. of combined salicylic acid; yet this mixture, in 15 hours at 40° C. digested but 17·10 per cent. of fibrin, while the same amount of the neutral trypsin solution digested 57·80 per cent.

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* Tested by tropæolin 00 according to the method of Danilewsky (Centralbl. med. Wiss., 1880). One drop of a solution containing 0·028 per cent. free salicylic acid gives a reddish-violet color, which is, however, not permanent as in the case of hydrochloric acid, but transient. With hydrochloric acid, one drop of a 0·003 per cent. solution will give the reaction.

Combined hydrochloric acid has a greater hindering action than salicylic acid, as the following results show:

Pancreatic solution of trypsin.	Fibrin digested in 18 hours.
neutral	57·80 per cent.
0·034 per cent. combined HCl+no free HCl	3·90
0·034 " " HCl+0·005 per cent free HCl	2·31
0·034 " " HCl+0·010 " "	0·87

It is thus evident that in an ordinary digestive mixture, or even where albuminous matter is present only in limited quantity, the addition of hydrochloric or salicylic acid to a neutral solution of trypsin reduces its proteolytic action to a minimum before any free acid is present.

4.—*Influence of Bile, Bile Salts and Bile Acids on the Proteolytic Action of Trypsin.*

The addition of bile to a *neutral* pancreatic juice causes but little change in its proteolytic action, as is seen from the following results obtained with ox bile containing 8·3 per cent. solid matter:

Bile.	Weight of undigested residue.	Fibrin digested.
0 per cent	0·4118 gram.	59·82 per cent.
1·0	0·3907	60·93
10·0	0·3938	60·62

A slightly increased action is the only effect produced on the trypsin.* The addition of bile to an *alkaline* pancreatic juice does not produce any very different results. The following were obtained with a pancreatic juice containing 0·3 per cent. sodium carbonate and fresh ox bile containing 10·02 per cent. solid matter:

Bile.	Weight of undigested residue.	Fibrin digested
0 per cent.	0·3056 gram.	69·44 per cent.
0·25	0·3074	69·26
0·50	0·3488	65·12
1·00	0·3633	63·67
5·00	0·3278	67·22
10·00	0·3603	63·97

Here there is no increased proteolytic action, neither is there any very great retarding effect produced. Pure sodium glycocholate and taurocholate produce results similar to bile, as the following table shows. The pancreatic juice contained 0·3 per cent. sodium carbonate:

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* Compare Heidenhain, Pflüger's Archiv., vol. x, p. 579.

Sodium taurocholate.	Weight of undigested residue	Fibrin digested.
0 per cent.	0·2308 gram.	76·92 per cent.
0·05	0·2566	74·34
0·10	0·3048	69·52
1·00	0·2832	71·68
sodium glycocholate.		
0·10	0·2576	74·24
0·20	0·3154	68·46

The presence of 3·0 per cent. crystallized ox bile caused a somewhat different result, increasing the proteolytic action slightly; thus, while the control, containing 0·3 per cent. sodium carbonate, digested 88·69 per cent. of fibrin, the same trypsin solution plus 3 per cent. of crystallized bile digested in the same time 89·73 per cent. of fibrin.

While bile or bile salts have but little influence on the proteolytic action of trypsin, the bile acids, even small percentages, have a much more marked effect. The following results, obtained by the addition of the bile acids to a neutral pancreatic juice, show the extent of the action:

Bile acids.	Weight of undigested residue.	Fibrin digested.
0	0·2516 gram.	74·84 per cent.
Glycocholic, 0·03 per cent.	0·1993	80·07
Taurocholic, 0·10	0·3455	65·45
0·20	0·4332	56·68
0·50	0·4170	58·30

Here the retarding influence of taurocholic acid is very manifest, while, on the other hand, the small percentage of glycocholic acid appears to increase the action of the ferment.

In view of the possible acid-reacting character of the contents of the small intestines, it becomes an interesting point to ascertain the influence of bile on the action of trypsin in the presence of more or less combined acid. With a pancreatic juice in which the proteids were partially saturated with salicylic acid, 0·1 per cent. combined acid being present, the following results were obtained:

Bile.	Weight of undigested residue	Fibrin digested.
0 per cent.	0·4822 gram.	51·78 per cent.
1·0	0·4858	51·42
10·0	0·4091	59·09

This increased action in the presence of 10 per cent. of bile accords with Lindberger's results, this experimenter having found that bile in the presence of small percentages of (combined?) acetic and lactic acids tends to diminish the retarding effect produced by the acids alone.

In the presence of combined hydrochloric acid, the bile salts produced no effects whatever; the trypsin was entirely without action.

X.—ABSORPTION OF ARSENIC BY THE BRAIN. BY R. H. CHITTENDEN AND HERBERT E. SMITH, M.D.

SOME time since one of us* advanced the view that the amount of arsenic present in the brain, in cases of arsenical poisoning, is an index to the *form* in which the poison was taken, viz: whether in a readily soluble and diffusible form, such as sodium arsenite, or in a comparatively insoluble form, as arsenious oxide or aceto-arsenite of copper. The original experiments of Scolosuboff † on animals, with sodium arsenite, plainly showed the capability of nerve tissue for the absorption of arsenic; yet the recorded observations of toxicologists tend to show, as a rule, the presence of but traces of this metal in cases of arsenic poisoning, either acute or chronic. Scolosuboff's results are, however, undoubtedly correct; arsenic when taken in a very soluble and diffusible form without doubt does accumulate in the brain, but in our opinion *only when in that condition*, and thus in the more common forms of poisoning with the white oxide or other insoluble forms of arsenic, but a trace of the poison is to be found in the brain at any one time.

With a firm belief in the truth of the above statement, founded on personal experience and the recorded results of other workers in this field, it was maintained by one of us in a previous paper‡ that the presence of weighable amounts of absorbed arsenic in the brain may be taken as an indication of the administration of a soluble form of the poison. Experiments on animals tend to show the correctness of the theory and the results of toxical investigations, so far as our knowledge extends, contain nothing contrary to this view. If true, we ought never to find under any circumstances an accumulation of arsenic in the brain, after the administration of an insoluble form of the poison. Hence, the study of arsenic cases, where the form of poison is known, is of great importance in this connection.

We have had a recent opportunity of adding two more cases to the list of those which bear testimony to the truth of the above theory; two fatal cases of arsenic poisoning, one of which was caused by the white oxide, the other presumably by Paris green or aceto-arsenite of copper.

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* Chittenden, Amer. Chem. Jour., vol. v, p. 8; and Medico-Legal Journal, vol. ii, p. 237.

† Bulletin de la Société Chimique de Paris, vol. xxiv, p. 125.

‡ Chittenden, loc. cit.

Case A.—L. K., a middle-aged laboring man, ate for his dinner at noon a quantity of bean soup. Almost immediately after, he was seized with vomiting and purging, cramps in the legs, and all the ordinary symptoms of acute arsenic poisoning. There were no marked cerebral symptoms. At 9 P. M. of the same day the patient died in a condition of collapse, having thus lived nine hours after eating the poisoned soup. An autopsy made the following day by Dr. M. C. White of the Yale Medical School, to whose courtesy we are indebted for a description of the case, and also for the organs for analysis, revealed the following points of interest: "The mucous membrane of the stomach was very much inflamed, especially around the cardiac orifice. The duodenum was likewise much inflamed, also the lower part of the rectum, showing here as a red mottled congestion. The remaining portions of the intestines were normal. The brain showed marked congestion. The kidneys were normal in appearance, the urinary bladder was nearly empty, and the mucous lining somewhat reddened. The lungs were normal, except the lower half of the right one, which was a little congested. The heart normal; small fibrinous clot in the right ventricle." In order to draw deductions of any value from the distribution of arsenic in the body of the deceased we must know positively as to the form in which the poison was taken. Fortunately, we were able to obtain the residue of the soup eaten by the deceased. Microscopic examination of the sediment plainly showed octahedral crystals of arsenious oxide, and we were able to separate from a small portion of the solid residue 24 milligrams of the oxide. Plainly the soup was poisoned by simply mixing with it arsenious oxide in substance. As to the quantity of arsenic present in the soup we have the following data: 125 c. c., oxidized with hydrochloric acid and potassium chlorate, yielded 314·6 milligrams of arsenious sulphide, equal to 253·2 milligrams of arsenious oxide. As to the amount taken by the deceased we have no knowledge; we infer, however, since the soup constituted the main portion of his dinner, that a large quantity was eaten, which view we think is substantiated by the intensity of the vomiting and purging so characteristic of large doses of the poison.

Here then, we have an unquestionable case of poisoning with arsenious oxide, and under conditions most suitable for rapid absorption; a probable empty condition of the stomach, together with a large amount of the poison, a considerable portion of which must probably have been dissolved in the soup. Added to this, nine hours intervened between the taking of the poison and death. Certainly then everything favored an absorption of poison by the brain, if such is characteristic of this form of arsenic. Naturally the vomiting and purging would remove much of the poison, still the relative proportion of absorbed arsenic would not be materially altered, and thus if Scolosuboff's results with soluble arsenites are applicable to arsenious oxide, we ought to find in this case, in conformity with his results, a larger percentage of arsenic in the brain than in the liver or kidneys.

Following are the results actually found:*

Liver (1259 grams) contained 76·0 milligrams As_2O_3.
Kidney and bladder (332 grams) contained 0·6 milligram As_2O_3.
Brain ($\frac{1}{2}$=328 grams dry) contained simply a recognizable trace.

Case B.—J. G., a young woman, age unknown. Regarding the details of this case we have less definite knowledge. She was last seen alive on Friday night, at which time she threatened to poison herself. The following Monday morning she was found dead, and near her an open package of *Paris Green.* She had evidently been dead some time, and both the condition of her room and person gave evidence of excessive purging and vomiting. An autopsy by Dr. White showed an entire absence of inflammation of the alimentary tract, and also a lack of any abnormal condition sufficient to account for death. The verdict was therefore, death by poisoning with Paris green or aceto-arsenite of copper.

Through the kindness of Dr. White we were able to obtain portions of the body for analysis. The contents of the stomach were entirely free from arsenic, the poison having been wholly removed by the purging and vomiting; the trace found therefore, was the amount absorbed by the muscle tissue of the stomach. Following are the amounts of arsenic found in the parts analyzed:

Liver (2984 grams) contained 12·78 milligrams As_2O_3.
Kidneys and bladder (515 grams) contained 3·40 milligrams As_2O_3.
Muscle of thigh (735 grams) contained 0·97 milligram As_2O_3.
Stomach (425 grams) contained a trace.
Brain (1179 grams) contained a trace.

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* The method of analysis consisted in the oxidation of the tissue with nitric and sulphuric acids, the arsenic being weighed as metallic arsenic. See Amer. Chem. Journal, vol. ii, p. 235.

The trace of arsenic in both the muscle tissue of the stomach and in the brain was very small; the entire brain could not have contained more than 0·2 of a milligram of arsenic.

These results plainly substantiate the views set forth above and lend favor to the belief that Scolosuboff's results with sodium arsenite are applicable only to that form of poison, and not to the more insoluble compounds of arsenic. These two cases, therefore, are additional evidence that in poisoning with arsenic the presence of an appreciable amount of poison in the brain, is an indication amounting almost to proof positive of the administration of a soluble and diffusible form of arsenic

XI.—INFLUENCE OF POTASSIUM AND AMMONIUM BROMIDES ON METABOLISM. BY R. H. CHITTENDEN AND W. L. CULBERT, PH.B.

As a question in the physiology of nutrition, it is very desirable to be able to state something definite regarding the influence of bromides upon the metabolism of the body; particularly their influence upon the metabolism of proteid matter, as shown in the excretion of urea and uric acid, and in view of their special application as therapeutic agents in diseases of the nervous system, their influence also on the decomposition of nerve substance, as shown in the excretion of phosphoric acid. Two complete investigations appear to have been made upon this subject; one in 1868 by Dr. J. H. Bill,* and one in 1883, by Dr. B. Schulze,† the results of which are more or less in direct opposition to each other. We have also seen a reference to two other investigations, quoted by Dr. Wood,‡ in which Dr. Rabuteau found the daily excretion of urea slightly lessened under the influence of bromide, as did also Dr. Bartholow.

Dr. Bill's investigation, which was a very thorough one, had for one of its objects to ascertain whether bromides reduce the amount of phosphoric acid excreted, like such known hypnotics as morphine; at the same time careful examination was made of the variations in urea and uric acid under the different conditions of the experiments. The experiments were all conducted on one person with a body weight of 160 pounds, which remained fairly constant throughout. The experiments were made in series under known conditions with uniformity of habits, diet, etc. Unfortunately, however, no data are given regarding the nature of the diet, the closeness with which it was adhered to, or whether the body was kept in a state of nitrogenous equilibrium. Each series of experiments, moreover, covers at the most, but six days; three days without bromide and three days with, consequently slight variations might easily be absorbed in an average of three results. The urea in Dr. Bill's work was determined with a "mercury solution," phosphoric acid with uranium solution and uric acid by precipitation and weighing as such. The results obtained were as follows: With moderate doses of potassium bromide (3·0–8·0 grams per day) urea was not affected, phosphoric acid was slightly increased, uric acid likewise, though much more decidedly; with larger doses of bromide (10·0–14·0 grams per day, continued for 2–3 days) phosphoric acid was diminished in amount, but this Dr. Bill intimates could not be attributed to regular hypnotic action, since the other urinary constituents were likewise diminished, notably the urea. Both large and small doses of bromide increased the quantity of urine passed in the twenty-four hours. This Dr. Bill asserts was not due to the increased drinking of water, for no thirst, not even with the largest doses, was ever present.

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* Amer. Jour. med. Sciences, July, 1868. Experimental Researches into the action and Therapeutic value of Bromide of Potassium.

† Zeitschrift für Biologie, vol. xix, p. 301. Einfluss des Bromkalium auf den Stoffwechsel.

‡ Therapeutics, p. 337.

Dr. Schulze, experimenting on his own person, obtained results quite different from these. This investigator lived on a fixed diet of the following composition:

220 grams fresh meat =7·13 grams N.
50 grams air-dried wheat bread=0·92 grams N
30 grams cocoa powder =1·14 grams N.
30 grams butter.
30 grams sugar.
5 grams salt.
1500 grams water.

9·19 grams N.

The average amount of nitrogen excreted daily was about 11 grams.

Taking this amount of food daily, with uniform habits of sleep, exercise, etc., Dr. Schulze states that he soon reached a point where the daily excretion of nitrogen, sulphur and phosphorus remained fairly constant. Potassium bromide was then taken three days, in divided doses of 10 grams each day. Diuretic action was very noticeable. Phosphorus was diminished, sulphur very much increased, and nitrogen (on two days) apparently slightly increased under the influence of the bromide. As, however, the increased excretion of sulphur was not accompanied with a corresponding increase in the excretion of nitrogen, Schultze considers that the increase in sulphur cannot be due to increased metabolism of simple albuminous matter, and seeks to show that the potassium bromide must have decomposed, to a slight extent, some nitrogenous phosphorized principle or principles, such as lecithin (glycerine-phosphoric acid) and nuclein, so abundant in the brain and nerve substance in general. Schulze therefore concludes that under the influence of potassium bromide there is probably a decided diminution of metabolic activity in the nervous system, accompanied by decreased nervous irritability.

By determining the nitrogen of the fæces, Schulze concluded that the bromide exercised no particular influence on the digestibility of the food.

In our experiments great care was taken first, to insure body equilibrium and then to obtain sufficient data by analysis, to be sure of the requisite constancy in the composition of the daily excretions. The experiments were tried throughout on the person of one of us (W. L. C.), of good physique and vigorous constitution. The diet was weighed out each day with scrupulous care and was as follows:

Fresh meat [beef]	142·0	grams.
Potatoes	283·5	"
Wheat bread	256·0	"
Oat meal	50·0	"
Butter	56·7	"
Sugar	28·3	"
Salt	0·7	"
Milk	700·0	"
Water	345·5	"

This diet was commenced on the third day of April and continued for nine days before any attempt was made to ascertain the daily amounts of urea, etc., excreted. Then the urine was analyzed for nine successive days, after which doses of potassium bromide were taken. The above daily amount of food was divided into three portions and taken at the same time each day; at 7:30 a. m., 1 p. m., and 6 p. m. Exercise was taken regularly and in stated amounts; consisting of a walk each morning before breakfast and exercise with dumbbells just before retiring at 11 p. m. Care was taken not to exercise so freely as to induce perspiration. Throughout the day routine duties allowed of regular habits. It was thus found possible to keep even the minor conditions of the experiment constant throughout. The urine was collected from 7:30 a. m. of one day to 7:30 a. m. of the next, and was at once analyzed. Urea was determined by Pflüger's* modification of Liebig's method, with a standard solution of mercuric nitrate. All the precautions so carefully worked out by Pflüger; preparation of a mercuric nitrate solution of the proper specific gravity, a standard solution of sodium carbonate to neutralize the acid of the former, preliminary determination of chlorine and removal of the same, before precipitating the urea; were carried out with very satisfactory results. Uric acid was determined according to the older method of Heintz* with the modification of Zablins, and being conducted each time under exactly the same conditions and with a urine of approximately the same composition, the results are to be considered as strictly comparable. Chlorine and bromine were determined in the usual manner with a standard solution of silver nitrate; the results, however, are not given as they are of value only as essential to the urea determinations. Total phosphoric acid was determined by means of a standard solution of uranium nitrate.† Phosphoric acid in combination with alkali-earths was determined by precipitation with ammonium hydroxide, allowing the mixture to stand 24 hours, filtering the precipitated phosphates, washing thoroughly with diluted ammonia, then dissolving in a definite amount of dilute acetic acid and titrating with uranium solution. Total amount of solid matter contained in the 24 hours' urine, was calculated by the use of Christison's formula.

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* Pflüger's Archiv, vol. xxi, p. 248.

The diet specified, was commenced on the 3d day of April; on the 12th the urine was collected for analysis, the body weight taken and the investigation then carried forward without interruption. Table No. I. gives the results of the analysis of the urine for nine consecutive days, and shows the average amount of variation to be expected under the conditions of the experiment.

On the 21st of April, 60 grains of potassium bromide were taken in divided doses as seen in Table No. II. The bromide was taken about midway between the hours of eating, so that it might not affect digestion. On the 22d the dose was increased to 100 grains and then to 150, the latter amount being taken daily for three consecutive days. Table No. II. shows the effects of the bromide on the system, for the six days it was taken.

On the first day, the only apparent influence of the bromide is to cause a diminution in the amount of phosphoric acid excreted; seen both in the total P_2O_5 and in the P_2O_5 in combination with alkali-earths. On the second day, the diuretic action of the salt is apparent, accompanied with an increase in specific gravity, and a decided increase in the amount of urea excreted, together with a slight increase in the amount of uric acid. Phosphoric acid was still diminished in amount. On the third day, the body weight commenced to diminish and continued to do so throughout the experiment; diuretic action was still apparent as was also increased elimination of urea, and to a slight extent of uric acid likewise. Indeed, the most noticeable effect of the bromide, next to its diuretic action is its decided influence on proteid metabolism as shown by the increased elimination of urea. As to phosphoric acid the results are not so striking, although an average of the two series shows a diminished excretion, both of total P_2O_5 and of P_2O_5 in combination with alkali-earths. The average difference in the two series of results is clearly shown by the following table:

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* Die Lehre vom Harn, Salkowski und Leube, p. 94–95.

† Die Lehre vom Harn, p. 184.

	Average of Table	
	No. I, without KBr.	No. II, with KBr.
Total quantity of urine	926 c. c.	1010 c. c.
Sp. Gr.	1025, 8	1026, 3
Total solid matters	56·7329 grams.	63·6252 grams.
Total P_2O_5	2·7540	2·5426
P_2O_5 in combination with Ca and Mg	0·6022	0·5452
Uric acid	0·6752	0·6858
Urea	34·8681	35·9454

Our results therefore plainly indicate, that under the influence of potassium bromide, nitrogenous metabolism is increased while the excretion of phosphoric acid is slightly diminished in amount; not however, to any such extent as would be expected by an active hypnotic agent. The bromide taken, the largest doses about 10 grams per day, produced its usual physiological effects; such as drowsiness, diminution of the circulation with accompanying coldness and paleness of the skin. Constipation was not noticed while taking the bromide, but later on it became somewhat troublesome, once or twice alternating with a slight diarrhœa. In accord with Dr. Bill, we noticed an increase in the acidity of the urine while taking the bromide, as also a deepening of the color.

With bromide of potassium therefore, our results agree with those of Dr. Schulze in showing an increased excretion of nitrogen (urea and uric acid), although far more pronounced than he found in his experiments, while the diminution in phosphorus is less pronounced than found by Schulze.

With Dr. Bill's experiments our results agree in so far as the diminution of phosphoric acid is concerned, but are entirely different as regards the urea. Since, however, Dr. Bill retained uniformity in diet only during the days of the experiments, it is quite possible that lack of nitrogenous equilibrium may have had some influence on his results. The increased elimination of urea noticed in our experiments is certainly indicative of increased metabolic activity; it is, however, suggestive that potassium bromide has been recently found to exercise a very decided accelerating influence on the proteolytic action of both pepsin-hydrochloric acid* and trypsin;† while, therefore, this fact may have something to do with the increased elimination of nitrogen, particularly as the diet used is quite rich in proteid matter, it seems more probable to suppose that the above changes in the excretion of nitrogen are due rather to changes in the tissue proteids; still it would have been interesting if the nitrogen in the fæces had been determined each day.

Although the last dose of potassium bromide was taken on the 26th of April, the same diet was still continued and the urine carefully examined daily, until the 8th of May, at which time the amount of bromine in the urine was reduced to a minimum. Dr. Bill states that bromine usually disappears entirely from the urine in ten days after the last dose of bromide.

The results of the twelve days analyses are shown in Table No. III. In examining this table it is interesting to note how quickly the elimination of urea is changed on stopping the doses of bromide. On the 26th, the last day the bromide was taken, the excretion of urea amounted to 37·5 grams; on the 27th it fell to 31·8 grams, far below what it had been any time before the bromide was taken. It would thus appear that after withdrawal of the bromide, nutrition which had been accelerated, rebounded in proportion to the preceding acceleration. Uric acid, moreover, which had likewise been increased in amount by the bromide, was now also correspondingly diminished. Furthermore, the diuretic action of the bromide was at once stopped, and the specific gravity fell to 125·5. In the case of phosphoric acid, however, the action of the bromide appears to be continued for a day or two after its withdrawal, and indeed it is noticeable throughout, that the diminution in phosphoric acid excreted, is not at all proportional to the amount of bromide taken. In fact phosphoric acid, both total P_2O_5 and alkali-earth P_2O_5, appears to be more decidedly diminished on those days when the amount of bromide in the blood was the smallest, notably on the 21st, 24th and 27th of April. By the 3d day after withdrawal of the bromide, the excretion of urea had gone nearly back to the daily amount, prior to taking the bromide; still it is to be seen in Table No. III, that the average excretion of urea, uric acid and phosphoric acid is below the average excretion recorded in Table No. I. In fact after the continued doses

* Chittenden and Allen. Trans. Conn. Acad., vol. vii,

† Chittenden and Cummins. Ibid.

of potassium bromide, the metabolism of the body did not fall back to its original height; but being temporarily accelerated during the exhibition of potassium bromide, it [the nitrogenous metabolism] fell back on withdrawal of the same, to a far lower level, and although later somewhat increased in amount, its average was still lower than recorded in Table No. I. Nutrition had evidently been disturbed; the body weight showed gradual diminution, and on the 4th and 6th of May there was slight diarrhœa accompanied with a decided decrease in the amount of urea and uric acid excreted.

Dr. Bill appears to have experimented somewhat with sodium bromide, although we find no results recorded, aside from the fact that this salt, like potassium bromide, caused an increased excretion of uric acid, and the general statement that "when taken by the mouth, bromide of sodium does not produce the same effects as bromide of potassium." In view of the increased excretion of urea, noticed under the influence of the potassium salt, we were interested in seeing whether ammonium bromide would have a like influence, especially in view of the fact that v. Schrœder* has shown that ammonium carbonate is directly convertible into urea by passage through the liver.

The physiological action of ammonium bromide is stated to resemble in many points that of potassium bromide, while in other points it differs essentially.† As to its influence on metabolism no experiments whatever appear to have been made.

On the 9th of May, 75 grains of ammonium bromide were taken, in divided doses, as shown in Table No. IV. In all, 425 grains of the salt were taken in four consecutive days. The action of the salt on the system was not as pleasant as that of potassium bromide; causing a general weakness and indisposition, a slight diminution in the pulse, an occasional cold perspiration, more marked lividity of the countenance and a parched, dry taste in the mouth. An habitual eruption of the skin was moreover much increased and accompanied with acne on the back and shoulders. Undoubtedly these disagreeable symptoms were much augmented by the temporary lassitude which was beginning to be apparent; doubtless due to the approach of warm weather together with lack of the accustomed vigorous exercise and the long continued use of the somewhat monotonous diet.

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* Archiv f. exp. Pathol., vol. xv, p. 364. Also Report on Progress in Physiological Chemistry in Amer. Chem. Jour., vol. v, p. 219.

† Wood, Therapeutics, p. 341.

TABLE I.—WITHOUT BROMIDE.

April.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid.	Urea.
	grams.				grams.	grams.	gram.	gram.	grams.
12	72100	950 c. c.	Acid.	1026	59·0473	2·7678	0·6576	0·7443	35·7596
13	72100	930	Acid.	1026	57·2590	2·8596	0·6132	0·6901	35·0067
14	72000	1012	Acid.	1025	60·4227	2·7249	0·6060	0·6654	34·3699
15	72000	920	Acid.	1025	54·9298	2·7088	0·5587	0·6969	35·3247
16	72800	928	Acid.	1025	55·4074	2·7352	0·6188	0·7146	35·4567
17	72600	915	Acid.	1025·5	55·7510	2·6818	0·5946	0·7407	32·5379
18	72400	880	Acid.	1026·5	55·7755	2·6909	0·5569	0·5936	34·5360
19	72100	972	Acid.	1026	60·4147	2·9227	0·6399	0·6551	36·4960
20	72100	830	Acid.	1026	51·5887	2·7002	0·5746	0·5769	34·3255

TABLE II.—WITH POTASSIUM BROMIDE.

April.	Body weight	Total quantity Urine.	Reaction.	Sp Gr.	Total solid matters.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid	Urea.	Amount of KBr taken, in grams
21	grams 72200	870 c.c.	Acid.	1026	grams. 54·0749	grams 2·2169	gram. 0·5542	gram. 0·6895	grams 34·7129	20 at 10 a. m. 20 3 p. m. 20 10 p. m.
22	72200	1005	Acid.	1027	64·9316	2·5268	0·5805	0·7075	36·0992	33 at 10 a. m. 33 3 p. m. 34 10 p. m.
23	71700	1018	Acid.	1027	65·7715	2·6630	0·5411	0·6388	36·1708	50 at 10 a. m. 50 3 p. m. 50 10 p. m.
24	71600	920	Acid.	1025·5	56·0557	2·2876	0·4805	0·6118	35·1002	50 at 10 a. m. 50 3 p. m.
25	71500	1125	Acid.	1026·5	71·3039	2·9013	0·5096	0·6750	36·4778	50 at 10 a. m. 50 3 p. m. 50 10½ p. m.
26	71000	1120	Acid.	1026	69·6137	2·6605	0·6056	0·7924	37·5117	50 at 10 a. m. 50 3 p. m. 50 10 p. m.

TABLE III.—WITHOUT BROMIDE.

April	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid	Urea
27	grams. 70800	830 c. c.	Acid.	1025·5	grams. 50·5720	grams. 2·5779	gram 0·4527	gram 0·6420	grams 31·8277
28	70400	840	Acid.	1025·5	51·1813	2·5623	0·5008	0·6846	32·3742
29	70500	905	Acid.	1025·5	55·1417	2·7606	0·4810	0·7647	33·3858
30	70400	945	Acid.	1025·5	59·2743	2·5806	0·4996	0·6693	33·4907
May. 1	70400	985	Acid.	1024·5	57·6063	2·5676	0·5326	0·6368	34·3289
2	70500	1050	Acid.	1028·5	58·8438	2·5138	0·5435	0·6405	33·4344
3	70100	1020	Acid.	1024·5	59·6533	2·6682	0·5987	0·6324	33·8654
4	69800	835	Acid.	1025	49·8547	2·3155	0·4438	0·5716	30·6414
5	70100	905	Acid.	1024	51·8222	2·5849	0·4476	0·7353	33·1222
6	69600	905	Acid.	1028	49·6144	2·5682	0·4350	0·6149	31·9801
7	69600	830	Acid.	1026·5	52·6064	2·5625	0·4949	0·6536	34·2450
8	70000	930	Acid.	1025	56·5945	2·5102	0·5373	0·6738	31·5995

TABLE IV.—WITH AMMONIUM BROMIDE.

May.	Body weight	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid.	Urea.	Amount of $(NH_4)Br$ taken, in grains.
9	grams. 69700	1040 c. c.	Acid.	1024	grams. 59·5416	grams. 2·4514	gram. 0·4895	gram. 0·6630	grams. 34·2265	25 at 10 a. m. 25 3 p. m. 25 11 p. m.
10	69700	1130	Acid.	1024·5	66·0864	2·2248	0·6581	0·6690	33·3503	33 at 10 a. m. 33 3 p. m. 34 11 p. m.
11	70100	990	Acid.	1025	59·1092	2·5581	0·5470	0·6633	34·8876	40 at 2 p. m. 30 7 p. m. 30 11 p. m.
12	70400	1130	Acid.	1024	64·7062	2·8177	0·6051	0·7051	36·1377	50 at 2 p. m. 50 7 p. m. 50 11 p. m.

TABLE V.—WITHOUT BROMIDE.

May.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Total P_2O_5	P_2O_5 with Ca and Mg.	Uric acid.	Urea.
13	grams. 70400	970 c. c.	Acid.	1024·5	grams. 56·7291	grams. 2·3746	gram. 0·4329	gram 0·6936	grams. 33·5846
14	70400	835	Acid.	1025·5	50·8766	2·1505	0·4081	0·7156	31·2645

However this may be, the use of ammonium bromide in our experiments gave rise to more unpleasant symptoms than the use of like amounts of the corresponding potassium salt. Like the potassium salt, ammonium bromide caused increased acidity in the urine and a brighter color. As to its influence on metabolism, a study of Table No. IV, and comparison with No. III, plainly shows a decided accelerating influence on the excretion of urea; the average of the results, moreover, shows a very slight diminution in the excretion of total phosphoric acid, at the same time it would appear, in accord with what was observed with potassium bromide, that the diminution was greatest with the smallest amounts of bromide, as on May 9th and 10th and on the 13th and 14th after withdrawal of the bromide. With the largest amount of ammonium bromide, on the other hand, phosphoric acid appeared to be increased in amount, thus according with what Dr. Bill observed with like quantities of potassium bromide.

The average difference in the two series of results is shown by the following table:

	Average of	
	Table No. III without $(NH_4)Br$.	No. IV with $(NH_4)Br$.
Total quantity of urine	915 c. c.	1072 c. c.
Sp. Gr.	1024, 8	1024, 4
Total solid matters	54·3970 grams.	62·3608 grams.
Total P_2O_5	2·5643	2·5130
P_2O_5 in combination with Ca and Mg	0·4972	0·5749
Uric acid	0·6599	0·6751
Urea	32·8579	34·6505

It is thus seen that diuretic action is even greater with the ammonium salt than with potassium bromide; likewise the excretion of both urea and uric acid is greater under the influence of ammonium bromide than in the case of the potassium salt; as to phosphoric acid the table of averages shows practically nothing, but as before observed a study of the individual results does indicate some action of the salt, although diminution in the excretion of phosphoric acid under the influence of ammonium bromide cannot be so surely claimed as with the potassium salt.

After withdrawal of the ammonium bromide, the urine was examined for two days more; the results showing the same, or even greater drop in the excretion of urea, observed under like conditions with the potassium salt (Table No. V.). Hence, so far as our experiments extend, the influence of the two salts on the metabolism of the body is very much alike, differing only in extent of action; the ammonium salt, as might be expected, causing the largest excretion of urea, not, however, necessarily from any greater influence on proteid metabolism, but merely as furnishing a certain amount of ammonia to be excreted as urea. Finally, our results, with both salts, fail to show that diminution in the excretion of phosphoric acid to be expected from active hypnotic agents, and in this respect, therefore, our results show nothing antagonistic to Dr. Bill's conclusion "that bromide of potassium in its legitimate action, is an anæsthetic to the nerves of the mucous membranes and a depressor of their action. Its hypnotic effects are secondary."

XII.—INFLUENCE OF CINCHONIDINE SULPHATE ON METABOLISM.

By R. H. CHITTENDEN AND HENRY H. WHITEHOUSE, PH.B.

WHILE much attention has been paid to the physiological study of the cinchona alkaloids; quinine particularly having been experimented with by several observers, to ascertain its influence on the metabolism of the body; we have not been able to find any recorded statements bearing on the action of the closely related alkaloid, cinchonidine. In physiological action, quinine is taken as a type of the group; cinchonine is stated* to be similar to quinine but less powerful, and that its history in the organism is parallel with that of quinine; cinchonidine is likewise stated to be weaker than quinine and in physiological action, apparently its equivalent when taken in doses one-third larger. Presumably, therefore, its action on the metabolism of the body is similar to that of quinine, although apparently no attempt has been made to determine this point. In view of the special action of quinine on nitrogenous metabolism, we have devoted our attention mainly to a study of the influence of cinchonidine on the excretion of urea, uric acid, phosphoric acid and chlorine. The experiments were conducted wholly on the person of one of us (H. H. W.) under uniform conditions of diet, exercise, etc. The diet, weighed out accurately each day, was composed of

255 grams meat (beef).
255 grams wheat bread
149 grams potatoes.
50 grams oat meal.
35 grams butter.
21 grams sugar.
570 grams milk.
350 grams water.

This diet, divided into three definite portions each day, was taken for some time previous to the experiments, so that the system might become accustomed to it and the metabolism of the body brought to a constant point. The body weight was determined each morning; the urine collected from nine A. M. of one day to nine A. M. of the next, making the 24 hours' urine, the analysis being made the same day. Urea was determined by Pflüger's* modification of Liebig's method, chlorine being removed by a standard solution of silver nitrate. Chlorine was determined by evaporating 10 c. c. of the urine with a weighed amount of potassium nitrate in a platinum crucible, igniting until the organic matter was completely removed, dissolving in water, acidifying with nitric acid, neutralizing with calcium carbonate and then titrating with silver nitrate solution. Uric acid was determined by Heintz's method as modified by Zablins† and phosphoric acid with a standard solution of uranium nitrate.‡ The amount of solid matter was calculated by the use of Christison's formula.

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* H C. Wood, Therapeutics, p. 81.

After taking the above diet for some time, the urine was analyzed for seven consecutive days prior to the exhibition of cinchonidine. The results, seen in Table No. I, show a very close agreement in the daily excretions.

On the 11th of May the first dose of cinchonidine sulphate was taken. The alkaloid salt was a finely crystallized preparation obtained from Powers and Weightman. The daily dose was usually divided into three portions and taken in tiny gelatine capsules, about five hours apart. In view of the fact that cinchonidine is a weaker alkaloid than quinine, it was not deemed necessary to try the influence of very small doses; on the first day, therefore, 15 grains of the salt were taken; on the second day 21·8 grains; on the third day 35·1 grains; and on the fourth 50 grains, making a total of 121·9 grains of cinchonidine sulphate in four consecutive days.§ The results of the analyses of the four days' urine, as well as those of the three following days, on which no cinchonidine was taken, are shown in Table No. II.

Comparing these results with those in Table No. I, and in Table No. III, it is seen that cinchonidine exercises a very decided influence on the nitrogenous metabolism of the body. Urea is at once affected: its excretion on the first day even, is diminished 6 per cent., while on the second day it is diminished 10 per cent., and on the fourth day when the largest dose of cinchonidine was taken, the excretion of urea was 16 per cent. less than in the normal urine. The influence

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* Pflüger's Archiv, vol. xxi, p. 248.

† Die Lehre vom Harn, Salkowski and Leube, p. 94–95.

‡ Die Lehre vom Harn, p. 184.

§ While taking the larger doses of cinchonidine, an intense ringing in the ears was temporarily experienced (cinchonism) together with partial deafness and slight dizziness. On one or two occasions a slight nausea was felt.

TABLE I.—NORMAL URINE.

May.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Chlorine.	Total P_2O_5.	Uric acid.	Urea
4	grams. 59400	975 c. c.	Acid.	1027·5	grams. 64·191	grams. 6·082	grams 2·586	gram. 0·774	grams. 40·801
5	59400	950	Acid.	1028	63·713	5·553	3·044	0·797	42·684
6	59200	905	Acid.	1027	58·471	5·493	2·708	0·721	41·877
7	59600	990	Acid.	1027	63·962	6·510	2·710	0·819	42·999
8	59600	1077	Acid.	1026·5	68·262	6·324	2·810	0·768	41·464
9	59600	1055	Acid.	1026·5	66·867	6·166	3·219	0·749	41·640
10	59600	1052	Acid.	1026	65·387	6·149	3·040	0·764	41·985

TABLE II.—SHOWING THE EFFECTS OF CINCHONIDINE.

May	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters	Chlorine	Total P_2O_5	Uric acid	Urea	Am't Cinchonidine sulphate taken
	grams				grams.	grams	grams.	gram.	grams.	grams.
11	59600	920 c. c.	Acid.	1027	59·440	5·044	2·776	0·713	39·715	15
12	59900	975	Acid.	1026·5	61·797	5·886	2·693	0·779	37·967	21·8
13	60000	1195	Acid.	1024	68·428	7·435	2·529	0·792	38·322	35·1
14	60000	1022	Acid.	1024	58·522	5·855	2·128	0·575	35·115	50
15	60200	1030	Acid.	1023	56·467	6·725	1·795	0·598	35·481	0
16	59400	950	Acid.	1026·5	60·212	6·028	2·390	0·666	35·989	0
17	58800	975	Acid.	1027·5	64·191	5·586	2·801	0·711	39·856	0

TABLE III.—SHOWING THE EFFECTS OF CINCHONIDINE

May	Body weight	Total quantity Urine	Reaction	Sp. Gr.	Total solid matters	Chlorine	Total P_2O_5	Uric acid	Urea	Am't Cinchonidine sulphate taken.
18	grams 59600	900 c. c.	Acid.	1028·5	grams. 61·468	grams. 4·879	grams 3·227	gram 0·701	grams 40·279	grams 0
19	59600	920	Acid.	1028·5	62·834	5·270	3·019	0·744	43·458	0
20	59000	950	Acid.	1029·5	67·225	5·735	3·109	0·893	43·354	0
21	59000	875	Acid	1030	62·997	4·851	3·035	0·699	40·999	50
22	59500	1047	Acid.	1027·8	69·704	6·708	2·674	0·705	40·407	45·8

TABLE IV.—SHOWING AVERAGE RESULTS.

	Table I. Normal urine.	Table II, with Cinchonidine.	Table II, 3 days following the last dose of Cinchonidine.	Table III, without Cinchonidine.	Table III, with Cinchonidine.
Total quantity urine	1001 c. c.	1028 c. c.	985 c. c.	923 c. c.	961 c. c.
Sp. Gr.	1027	1025·4	1025·6	1028·8	1029
Total solid matters	64·408 grams.	62·047 grams.	60·290 grams.	63·842 grams.	66·351 grams.
Chlorine	6·040	6·055	6·113	5·295	5·780
Total P_2O_5	2·874	2·532	2·329	3·118	2·855
Uric acid	0·770	0·715	0·658	0·779	0·702
Urea	41·421	37·780	37·109	42·362	40·703

of cinchonidine on the elimination of urea continues to be felt after discontinuing the use of the alkaloid; thus even three days after the last dose of cinchonidine, the elimination of urea is 6 per cent. less than in the normal urine. On the fourth day it is nearly back to the normal amount [see Table No. III.], and on the fifth and sixth days the excretion of urea rises somewhat above the average. Uric acid does not appear to be correspondingly affected. It is only under the influence of the largest doses, or rather under the long continued action of the alkaloid that the excretion of uric acid is diminished; thus on May 14th, when the final dose of 50 grains of cinchonidine sulphate was taken, the excretion of uric acid was for the first time diminished; its diminution, however, was very perceptible and it continued for the two succeeding days, after withdrawal of the alkaloid salt. Phosphoric acid was greatly diminished in amount under the influence of cinchonidine; the diminution commencing to show, as in the case of urea, with the first dose of the alkaloid salt, then gradually increasing in amount with increase in the dose of cinchonidine until maximum diminution was reached on the day after the final dose of the alkaloid. The alkaloid salt, moreover, appears to have had a slight diuretic action.

On the 21st of May, cinchonidine sulphate was again taken, the results of the analyses of the 19th and 20th showing that the urine had returned to its normal composition. Accordingly, 95·8 grains of the salt were taken on the 21st and 22d, producing the same results (Table No. III.) as noticed in the first series, viz: diminution in the amount of urea and uric acid excreted, a like diminution in the amount of phosphoric acid and an increase in the total amount of fluid.

Table No. IV. shows the *average* results under the different conditions of the experiments.

It is evident then, that cinchonidine has the power of lessening very materially the elimination of nitrogen, i. e. the consumption of tissue.

Ranke* first pointed out the retarding influence of quinine on the elimination of uric acid. Zuntz found by experiment upon himself that 25 grains of quinine reduced his elimination of urea nearly 40 per cent. A like diminution in the excretion of urea, under the influence of quinine, was noticed by Rabuteau in experiments upon dogs and also by Hermann von Boeck.† The most interesting experiments, however, with quinine are those carried out by Dr. G.

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* Quoted from Dr. H. C. Wood, Therapeutics, p. 74.

† Zeitschrift fur Biologie, vol. vii, p. 422.

Kerner,[*] and more recently by Dr. Prior[†] and Dr. Sassetzky.[‡] In Dr. Prior's article is to be found a very complete account of the literature of the subject. Dr. Kerner found that taking 9·3 grains of quinine hydrochloride per day in divided doses, for 3 days, making a total of 27·9 grains, caused a diminution in the excretion of urea, amounting on an average for the three days to 12 per cent., while the excretion of uric acid under like conditions was diminished 54 per cent. Phosphoric acid, too, was diminished somewhat; on an average about 4 per cent. per day. Diuretic action was very slight.

With larger doses of quinine; 77·5 grains of the hydrochloride in divided doses during three days; diuretic action was quite pronounced, the average increase in volume, amounting to 200 c.c. Moreover, urea was diminished on an average 23 per cent. per day, uric acid 82 per cent. and phosphoric acid 15 per cent. Oppenheim,[§] however, by a dose of 30·8 grains of quinine found an increase in the daily excretion of urea amounting to 4 grams, and he considers that Kerner's results are due to diminution of digestive action. It is certain that both quinine and cinchonidine do interfere with the proteolytic action of the gastric and pancreatic juices,[‖] but this retarding action can hardly be taken as explaining in full, the results obtained by Kerner or those obtained by us with cinchonidine.

Prior, moreover, by very carefully conducted experiments with quinine, has completely corroborated Kerner's results and has shown in addition, by a daily determination of nitrogen in the fæces, that diminution of urea and uric acid is not due to lack of digestive action, as suggested by Oppenheim. Prior's results show on an average, without reference to the size of the dose, the following effects of the quinine.[¶]

Quantity of urine.	Urea.	Uric acid.	Sodium chloride.	Sulphuric acid.	Phosphoric acid.
increase	decrease	decrease	decrease	decrease	decrease
10·65 per cent.	19·60 %	72·29 %	9·06 %	33·70 %	23·38 %

Sassetzky's results with fever patients, also corroborate Kerner's statements.

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[*] Pflüger's Archiv, vol. iii, p. 104.

[†] Ueber den Einfluss des Chinin auf den Stoffwechsel des gesunden Organismus. Pflüger's Archiv, vol. xxxiv, p. 237.

[‡] Ueber den Einfluss fieberhafte Zustände und Antipyretischer Behandlung auf den Umsatz der stickstoffhaltigen Substanzen und die Assimilation stickstoff-haltiger Bestandtheile der Milch. Virchow's Archiv, vol. xciv, p. 485.

[§] Pflüger's Archiv, vol. xxiii, p. 476–477.

[‖] Chittenden and Allen; Chittenden and Cummins. Trans. Conn. Acad., vol. vii.

[¶] Pflüger's Archiv, vol. xxxiv, p. 263.

v. Boeck considers that quinine owes its retarding influence on proteid metabolism to a direct action of the alkaloid upon the cells and their activity, although the alkaloid doubtless does unite with albumin or alter its constitution so as to render it less readily decomposable. Metallic salts, as lead and mercury, certainly form compounds with albumin difficultly decomposable, as also does arsenic, and v. Boeck suggests that these metallic poisons unite with the proteid matter of the various organs of the body, while quinine unites simply with the circulating albumin, explaining in this manner the ready elimination of quinine as compared with the slow excretion of mercury or arsenic, the latter of which v. Boeck* has shown has little if any influence on proteid metabolism. Prior, moreover, states that diminution in the urine of the end-products of nitrogenous metabolism is due, not to hindering of their excretion, but to actual hindering of their formation.

Comparing now Kerner's results, with the results obtained by us with cinchonidine, we see great similarity of action but decided difference in extent, particularly so far as the excretion of uric acid is concerned. With cinchonidine, the greatest average daily diminution in uric acid amounts to but 15 per cent., and this after taking about 121 grains of the alkaloid during four consecutive days. Selecting the lowest single result, that obtained on the day 50 grains of cinchonidine were taken and comparing the diminution then, with the average normal excretion, it is seen to amount to but 26 per cent. In the case of urea and phosphoric acid, the divergence is not so great; thus for urea the average daily diminution was 11 per cent. for the three days following the last dose of cinchonidine, while the greatest diminution noticed in any one day was 16 per cent.; with phosphoric acid the average diminution for the same period amounted to 19 per cent., while the greatest diminution noticed any one day was 38 per cent.; a diminution which at no time was reached in Kerner's experiments with quinine.

Thus in drawing a comparison between Kerner's results with 77·5 grains of quinine distributed through three days, and our results with 121 grains of cinchonidine extended over four days, we see two striking points of difference; with quinine there is a diminution in the amount of uric acid excreted of 82 per cent., with cinchonidine an average diminution of but 15 per cent.; with quinine there is a diminution of phosphoric acid amounting to 15 per cent., with cinchonidine, on the other hand, a diminution of 19 per cent. Hence it is to be seen that cinchonidine has a far less pronounced specific action on the excretion of uric acid than quinine, while on the other hand, diminution of phosphoric acid is much more pronounced with cinchonidine than with quinine. In Prior's experiments, however, with quinine, diminution of phosphoric acid is more pronounced.

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* Zeitschrift für Biologie, vol. vii, p. 430.

Is this diminution in the excretion of phosphoric acid under the influence of cinchonidine to be attributed simply to decrease of proteid metabolism, or is it in part due to a special action of cinchonidine on the metabolism of some phosphorized principles, presumably those of nerve tissue? If due to general decrease of proteid metabolism, we might expect to find that the addition of any non-nitrogenous principle to our fixed diet, whereby the decomposition of albuminous matter would be diminished, would cause a corresponding decrease in the excretion of phosphoric acid, or in other words that diminution of urea and phosphoric acid excreted, would be in the same ratio as noticed under the influence of cinchonidine.

This question we have endeavored to answer by a study of the influence of pure glucose on the elimination of urea, uric acid, and phosphoric acid, under the same conditions of diet etc., as observed in the experiments with cinchonidine.

The influence of carbohydrate food on proteid metabolism has been illustrated in many ways by various investigators, but so far as we know, no experiments with pure glucose have ever been tried. Through the courtesy of Dr. Arno Behr, of Chicago, we have been supplied with an abundance of chemically pure anhydrous glucose, which we have used in the following experiment. Before taking the sugar, the urine was analyzed for ten consecutive days, to insure an accurate average of the normal excretion under the conditions of the experiment. The results are shown in Table No. V.

200 grams of glucose were then taken daily in addition to the fixed diet, for nine consecutive days. The effect on the excretion of urea, etc., is shown in Table No. VI. At no time was sugar to be detected in the urine by Trommer's test. A comparison of the two tables shows the usual effects of carbohydrate matter on the excretion of nitrogen, viz : a diminution in the amount of both urea and uric acid. The volume of the fluid excreted, appears to be considerably lessened by taking the glucose. The excretion of phosphoric acid is likewise diminished.

TABLE V.—NORMAL URINE.

Day of month	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Chlorine	Total P_2O	Uric acid	Urea.
1	grams. 59400	1045 c. c.	Acid.	1020	grams. 72·658	grams 6·636	grams. 3·220	grams 1·060	grams. 46·281
2	59600	1060	Acid.	1028·5	72·395	7·030	3·249	1·068	46 463
3	59400	1070	Acid.	1028	71·761	6·194	3·333	0·802	46·607
4	59200	1042	Acid.	1029	72·450	6·090	3·167	0·907	45·770
5	60000	960	Acid.	1028	64·384	6·258	2·886	0·928	43·161
6	59800	990	Acid.	1028·5	67·615	6·399	2·659	0·804	44·078
7	59800	1004	Acid.	1026	62·404	6·320	2·621	0·722	44·000
8	59800	1090	Acid.	1027	70·423	7·780	3·304	0·715	47·103
9	59400	1073	Acid.	1027·5	70·643	8·141	3·045	0·921	45·900
10	59400	970	Acid.	1028	65·045	6·978	2·597	..	45·390

Day of month	Body weight.	Total quantity Urine.	Reaction	Sp. Gr	Total solid matters.	Chlorine.	Total P_2O_5.	Uric acid	Urea	Am't of Glucose taken
	grams.				grams	grams	grams	gram.	grams	grams
11	59600	985 c. c.	Acid.	1028·5	63·858	7·200	2·700	0·664	43·588	200
12	59500	900	Acid.	1030	64·797	6·727	2·781	0·721	42·114	200
13	59300	865	Acid.	1029	60·143	6·028	2·731	0·693	40·778	200
14	59800	840	Acid.	1028·5	57·370	4·862	2·440	0·663	40·009	200
15	59900	970	Acid.	1028·5	66·249	6·433	2·916	0·795	40·070	200
16	60000	1047	Acid.	1028	70·219	7·120	3·130	0·850	42·502	200
17	59600	950	Acid.	1028	63·713	6·674	2·832	0·642	40 887	200
18	59800	970	Acid.	1028	65·055	6·106	2·654	0·787	39·800	200
19	60000	965	Acid.	1026·5	61·163	5·610	2·527	0·684	38 752	200
20	60200	1072	Acid.	1026	66·630	7·290	2·934	0·846	41·740	0
21	59600	1014	Acid.	1026	63·025	6·553	2·655	0·809	39·835	0
22	60000	1076	Acid.	1026	66·879	6·289	3·108	0·901	45·439	0
23	59000	1036	Acid.	1026	65·636	6·350	2·962	0·891	44·492	0

These points of difference are all shown more clearly in the following table of averages :

	Average of	
	Normal urine	Urine under the influence of glucose.
Total quantity urine	1030 c. c.	938 c. c
Sp. gr.	1027·9	1028
Total solid matters	68·97 grams.	63·62 grams
Chlorine	6·78	6·31
Total P_2O_5	3·00	2·75
Uric acid	0·88	0·72
Urea	45·47	40·94

Coming now to the point at issue, viz: the relation between the amounts of urea and phosphoric acid excreted, we find that under the influence of glucose the average diminution of urea amounts to 10 per cent., while the average diminution of phosphoric acid under the same conditions is 8·34 per cent.

With cinchonidine, on the other hand, the average diminution of urea amounts to but 8·8 per cent., while the average diminution of phosphoric acid under like conditions is 11·9 per cent. Or, if we take the average of the three days following the last dose of cinchonidine, when both urea and phosphoric acid reach their maximum diminution, and compare these results with the average of the normal excretion we see that while the diminution of urea amounts to 10·4 per cent., the average diminution of phosphoric acid is raised to 18·97 per cent. Consequently, it would appear that while cinchonidine lowers the rate of decomposition of proteid matter in the body, it also has an effect upon the decomposition of some phosphorized principles, that being the only plausible explanation of the increased diminution of phosphoric acid noticed under the influence of the cinchonidine salt.

XIII.—THE POST-MORTEM FORMATION OF SUGAR IN THE LIVER, IN THE PRESENCE OF PEPTONES. BY R. H. CHITTENDEN AND ALEXANDER LAMBERT, B.A., PH.B.

CLAUDE BERNARD'S discovery in 1848, that the liver contains sugar, both before and after death, led at once to the inquiry as to the source of the sugar. This was apparently answered by Bernard's later discovery of glycogen, an amylaceous body readily convertible into sugar by acids and various ferments. Thus, Bernard's theory that the liver sugar resulted exclusively from glycogen has long been an accepted fact. In 1880, however, Seegen and Kratschmer in the first of a series of investigations,* state that the sugar formed in the liver does not have its origin, as supposed by Bernard, *wholly* in glycogen but that it is undoubtedly formed *in part* from other material. In a later communication† the same investigators show, in corroboration of their previous statement, 1. that the amount of sugar in the liver is increased very rapidly after death, in one case nearly 50 per cent. of the entire amount being formed within 10 minutes, while the whole process comes to an end inside of 24 hours; 2. that the glycogen formed in the liver is much more resistant to ferment action than has hitherto been supposed and that consequently the post-mortem formation of sugar by the action of a ferment upon glycogen could not take place so rapidly as the above. Moreover, direct experiments with dogs and with rabbits showed that in the first few hours after death, there was but little if any diminution in the amount of glycogen. Hence, Seegen and Kratschmer claim that the amount of glycogen remaining essentially the same, while the amount of sugar is greatly increased, tends to show conclusively that the liver sugar must be formed from some other material than glycogen and they venture the opinion that this source, whatever it may be, furnishes all of the liver sugar.

Boehm and Hoffmann,‡ however, take exception to the views of Seegen and Kratschmer, claiming possible analytical inaccuracies from the methods of procedure. They show, moreover, by experiments on cats and dogs, that after death, contrary to the statements of Seegen, increased formation of sugar is attended with a corresponding decrease of glycogen, at least within such limits as are incident to the errors of experiment; further that in the case of a cat's liver 32 per cent. of the liver glycogen disappeared in 24 hours after death, thus indicating less resistance to the action of ferments than would be implied by Seegen's and Kratschmer's results.

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* Ueber Zuckerbildung in der Leber. Pfluger's Archiv, vol. xxii, p. 236.

† Pfluger's Archiv, vol. xxiv, p. 467.

‡ Ueber die postmortale Zuckerbildung in der Leber. Pfluger's Archiv, vol. xxiii, p. 205.

In a later investigation,* Seegen shows that pieces of finely divided liver, kept in contact for an hour or longer with a solution of peptone yield a larger amount of sugar and even of total carbohydrates, than equal weights of the same liver under like conditions of treatment, without peptones. These results were obtained with the livers of calves, rabbits and dogs. Seegen, therefore, concludes that the liver is capable of forming from peptones, sugar and carbohydrates which are convertible into sugar.

A study of the analytical data plainly shows that the increase in sugar and total carbohydrates in the presence of peptone, although pronounced, is not great. The following experiment† with a calf's liver obtained from the market shows the most marked increase.

		With peptone.		Without peptone.	
No.	Time of the experiment.	Sugar.	Total carbohydrates.	Sugar.	Total carbohydrates.
I.	30 minutes	3·84 %	9·52 %	3·40 %	8·8 %
II.	48 hours	3·56	8·92	3·70	8·6
III.	96 "	2·66	8·00	2·82	7·8

Here the increase in total carbohydrates is seen to be only 0·72 per cent. and of sugar only 0·44 per cent. after 30 minutes. In Nos. II and III, longer standing in contact with the peptone tends to reduce the amount of sugar and to diminish the increase of total carbohydrates. This is attended with increase of acidity and Seegen considers that a portion of the sugar is decomposed in this long contact with peptone with formation of acid.

In a still later communication,‡ Seegen reports the results of other experiments tending to confirm his theory of the formation of carbohydrate matter from peptones in the liver. Thus, by feeding peptones to dogs, Seegen found that the content of sugar in the livers of eight dogs was considerably greater that in the normal liver, taking for the latter value the average of a number of determinations.

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* Die Einwirkung der Leber auf Pepton. Pfluger's Archiv, vol. xxv, p. 165.

† Pflüger's Archiv, vol. xxv, p. 171.

‡ Pepton als Material für Zuckerbildung in der Leber. Pflüger's Archiv, vol. xxviii, p. 99.

Likewise, by the injection of peptone solutions directly into the portal circulation of dogs, Seegen found the amount of sugar in the liver increased two and even nearly three times above the normal amount. Lastly, by warming portions of freshly excised liver at 40° C., with a solution of peptone in water and some fresh, defibrinated blood, through which a constant current of air was made to pass, the amount of both sugar and total carbohydrates was considerably greater than under like conditions, but without peptones. The following experiment* taken from Seegen's account, illustrates the average increase of carbohydrates under this method of treatment.

Two portions of a dog's liver taken 15 minutes after death, were mixed with 50 c. c. of water and 50 c. c. of defibrinated blood. To one portion 5 grams of peptone were added and air passed through the mixture for 5 hours. Following are the results obtained in both:

Wt of portion of liver	Method of treatment.	Liver sugar.	Total carbohydrates.	Glycogen.
40 grams.	without peptone and blood,	3·04 %	6·9 %	2·12 %
40 "	with peptone and blood,	3 87	8·4	2·02

Other experiments indicated that peptones themselves are without diastatic action and that the blood and air (to form oxyhaemoglobin) are by themselves without influence on the liver. Hence Seegen concludes that the liver cells, retained in a living condition by the action of blood rendered arterial by a current of air, are capable of forming from peptone more or less sugar; thus establishing, if true, that the animal organism is able to form carbohydrates from albuminous material.

This is certainly a very important question, for if Seegen's views are correct they overthrow the long accepted belief in the origin of liver sugar in the hepatic glycogen. It is true that Bernard himself, before his discovery of glycogen, thought that the liver sugar originated in albumin and there have always been, up to the present time, difficulties in explaining the origin of liver carbohydrates on the dehydration theory alone. As is well known, a certain amount of glycogen is formed during a purely animal diet and in chronic cases of Diabetes the excretion of sugar is continued even on a pure albuminious diet. Moreover, the suggestion has been before made that peptones in their passage through the liver undergo change. Thus Plosz and Gyergyai† noticed that while considerable peptone was to be found in the blood of the mesenteric veins and more or less in the liver, only the merest trace was to be found in the blood of the hepatic vein, indicating thereby a decomposition of peptone in its passage through the liver.

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* Pflüger's Archiv, vol. xxviii, p. 123.

† Ueber Peptone und Ernahrung mit denselben. Pfluger's Archiv, vol. x, p. 536.

Maydl* claims that since the products of the decomposition of all forms of glycogen are the same, it follows that the glycogens themselves are all identical, and since it is extremely improbable that the various carbohydrates with their different chemical constitutions should give *one* glycogen, he argues that it all must come from one source, viz: albumin.

This is not the place, however, to discuss the relative merits of the dehydration and storage theories, it is enough simply to understand that the possible origin of liver sugar in proteid matter is one which would make clear many hitherto unexplained points. The great obstacle, has been to understand where and in what manner the liver sugar could be so formed. Seegen's views therefore are of great importance, and are, moreover, in no sense, wholly inconsistent with previous ideas, but the question at once suggests itself whether the analytical data on which they are founded are sufficient to warrant their adoption.

The determination of sugar in organic fluids is not without difficulty, and where slight variations in results may cause differences of half a per cent. or more, it becomes an extremely delicate matter to determine how far such results shall be trusted. Consequently, whatever may be said as to whether the formation of sugar in the manner indicated by Seegen is a natural or an artificial process, we need first of all to know positively whether the liver under any circumstances is able to form sugar or other carbohydrate matter from peptones. This all hinges on the accuracy of Seegen's results, obtained by warming portions of liver with peptones. If an increase of sugar and total carbohydrates is found in the presence of peptone, then we must conclude that the latter has at least some influence on the formation of the liver sugar. Recent experiments† have plainly shown that neutral peptone has a stimulating influence on the amylolytic action of ptyalin of saliva and diastase of malt; both of these ferments convert more starch into sugar in the presence of peptone than without and it is natural to suppose that the presence of peptone would similarly affect the amylolytic ferment which presumably acts upon glycogen. Seegen's results, however, appear to show that while sugar is increased in the presence of peptone, glycogen remains nearly stationary, or if diminished, not at all in proportion to the increase in sugar. Boehm and Hoffmann, however, found the liver glycogen much less resistant and that its decrease was in proportion to the increase in sugar. Delprat,* too, came to similar conclusions and could obtain no proof whatever, of the correctness of the views advanced by Seegen and Kratschmer. We have, therefore, in view of the importance of the subject, undertaken a study of the question in the hopes of throwing some additional light upon the matter. In this, however, we have limited ourselves entirely to a study of the post-mortem formation of sugar and carbohydrates by the liver in the presence of peptones.

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* Zeitschrift fur physiol Chem., vol. iii, p. 196. Ueber die Abstammung des Glykogens.

† Trans. Conn. Acad., vol vi, p 343, vol. vii, p. 44.

Methods employed.

The animals experimented with, mainly rabbits, were killed by severing the jugular vein, the blood being collected and defibrinated. The liver was quickly taken out, the gall bladder removed and the liver then converted into a fine pulp by chopping, since it is probable, as v. Wittich has suggested, that glycogen is unequally distributed through the liver. Two equal portions of the sampled and finely divided liver were accurately weighed out and placed in separate flasks; one, with a solution of peptone and a known volume of blood, the other with an amount of distilled water equal in volume to that of the two former. Both were then placed in a bath and warmed at 38–40° C. for the time of the experiment. A continuous current of air was made to pass through the blood solution in order to render it arterial. At the end of the experiment, the mixtures were poured into boiling water and extracted as long as a trace of glycogen could be detected in the fluids, by the iodine test. This usually took about two days, working on an average with 40 grams of liver. At the beginning of the extraction, the tissue was generally boiled with 400–500 c. c. of water for about fifteen minutes and then filtered through a funnel plugged with absorbent cotton. By repeating this operation four or five times, the greater portion of glycogen could be removed, but a complete extraction could be obtained only by long continued boiling with fresh quantities of water or long heating on the water-bath, the tissue being ground up occasionally in a suitable mortar. The various filtrates were evaporated on a water-bath and finally united and made up exactly to 500 c. c., after which the extracts were filtered through dry paper filters to remove any traces of suspended matter which might have passed the cotton. Of these fluids, 200 c. c. of each were used for the determination of glycogen and sugar, and 200 c. c. also, for the determination of total carbohydrates.

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* Jahresbericht für Thierchemie, 1881, p. 321.

Determination of glycogen and sugar.—The 200 c. c. of fluid for the determination of glycogen and sugar were evaporated to a small bulk and then, when cool, precipitated by a large volume of alcohol. After standing 24 hours the clear supernatant fluid was filtered from the precipitated glycogen and peptones. The alcoholic filtrate and washings, containing the sugar, were then evaporated, the residue dissolved in water and made up to 100 c. c., in an aliquot portion of which the sugar was determined gravimetrically, by Allihn's* improved method.

The precipitate of glycogen, with its frequent admixture of peptone, was dissolved in water, the solution made up to 200 c. c. and then sufficient 10 per cent. hydrochloric acid added to make the solution contain 2 per cent. HCl. The mixture was then heated in a closed flask at 100° C. for 17 hours in order to convert the glycogen into dextrose, after which the solution was neutralized, concentrated somewhat, again made up to 200 c. c. and in an aliquot portion of this fluid, dextrose was determined by Allihn's method, from which was calculated the amount and percentage of glycogen. Delprat† states that in attempting to determine glycogen by Brücke's method he found the results considerably higher than when the isolated glycogen was converted into sugar by boiling with acid and the glycogen calculated from the data obtained. In our own experiments, the frequent presence of peptone prevented entirely the use of Brucke's method. 12 hours heating at 100° C., however, with 2 per cent. hydrochloric acid was found in our case insufficient to completely convert the glycogen into dextrose, while 17 hours was found amply sufficient for complete conversion and at the same time allowed no decomposition of the sugar formed. This is well illustrated by the following experiments:

A. 0·7665 gram pure, dried glycogen dissolved in 100 c. c. of water, was heated at 100° C. for 12 hours with sufficient hydrochloric acid to make the entire fluid contain exactly 2 per cent. The solution was neutralized, care being taken that the reaction did not become alkaline, then concentrated and finally made up to 50 c. c.

14 c. c. gave 0·4215 gram Cu=0·2251 gram dextrose=0·2025 gram glycogen.
14 " " 0·4233 " Cu=0·2263 " " =0·2036 "

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* Zeitschrift fur analytische Chemie, xxii, p. 448.

† Jahresbericht für Thierchemie, 1881, p. 322.

The 14 c. c. should have contained 0·2414 gram dextrose, the equivalent of 0·2146 gram of glycogen.

B. 0·6190 gram glycogen dissolved in 100 c. c. of water was heated at 100° C. for 17 hours in the presence of 2 per cent. of hydrochloric acid. Solution was then neutralized, evaporated and made up to 50 c.c.

18 c c gave 0·4585 gram Cu=0·2470 gram dextrose=0·2223 gram glycogen
18 " " 0·4555 " Cu=0·2454 " =0·2208 "

The 18 c. c. should have contained 0·2475 gram dextrose, equal to 0·2228 gram of glycogen. Hence, it is seen that 17 hours heating at 100° C. is needed for a complete conversion of glycogen into dextrose, which was the time invariably employed in the after experiments.

Influence of peptone on the conversion of glycogen into sugar by 2 per cent. HCl at 100° C.—The question naturally suggested itself in this connection whether the presence of peptone would interfere in any way with the complete conversion of glycogen into dextrose or whether the peptones by this long heating at 100° C. with the acid, would undergo any change by which reducing bodies might be formed and thus endanger the accuracy of the results. The latter point was tested by heating 2 grams of peptones in 100 c. c. of water containing 2 per cent. of hydrochloric acid for 17 hours at 100° C., at the end of which time no reduction at all could be obtained with Fehling's solution.

The first point was tested by the following experiment:

0·9290 gram of pure glycogen was dissolved in 100 c. c. of water, then 2 grams of peptone were added and sufficient acid for the solution to contain exactly 2 per cent. HCl, after which the mixture was heated at 100° C. for 17 hours. The solution was then neutralized, brought to a volume of 100 c. c. and the sugar determined.

10 c c. gave 0·1985 gram Cu=0·1017 gram dextrose=0·0915 gram glycogen.
10 " " 0·2025 " Cu=0·1039 " =0·0934 "

whereas in the 10 c. c. then should be present, according to calculation 0·1032 gram dextrose, the equivalent of 0·0929 gram of glycogen. Consequently the presence of peptone does not interfere with the accurate determination of glycogen by this method.

Influence of the presence of peptone on the determination of sugar by Allihn's method.—Seegen* finds that the volumetric determination of sugar with Fehling's solution is not materially affected by the presence of peptone. By repeated experiments we have convinced ourselves, that in the use of the gravimetric method, the presence of peptone may, unless certain precautions are taken, interfere slightly with exact determinations. With the Allihn method, variations of 2–5 milligrams in the amount of reduced copper are liable to occur if care is not taken in regulating the length of time the alkaline copper solution is heated after addition of the sugar solution. Under ordinary circumstances results most nearly in accord with theory are obtained by adding the sugar solution, as recommended by Allihn, to the previously heated Fehling's solution and then heating further until bubbles just begin to break upon the surface of the liquid. If heated longer, even only half a minute, a slight increase in the amount of reduced copper will generally be observed. Now whenever peptone is present to any extent in the sugar solution, we have found by experience that complete reduction does not take place quite so rapidly; the loss is not great, sometimes but a milligram or so, still the difference is appreciable. This, however, can be avoided by simply allowing the standard copper solution to boil for about 45 seconds after the addition of the sugar solution. Under such conditions, repeated trials have shown us, that the presence of peptone does not offer the slightest obstacle to accurate determinations of dextrose. Whenever, therefore, in the following experiments the solution to be tested contained peptone, the above rule has been invariably followed.

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* Pflüger's Archiv, vol. xxviii, p. 115.

Determination of total carbohydrates.—For this purpose 200 c. c. of the liver extract were heated in a closed flask at 100° C. with sufficient 10 per cent. hydrochloric acid to ensure a content of 2 per cent HCl, for 17 hours. The solution was then nearly neutralized, care being taken that the fluid did not become alkaline, concentrated and finally brought to a volume of 200 c. c., in an aliquot portion of which the total carbohydrates in the form of dextrose were determined in the usual manner. Seegen* states that in the determination of total carbohydrates, the fluid, after heating with acid, always became very dark, which occasionally interfered somewhat with the determination of sugar. Delprat,† however, states that in his experiments the solution, under like conditions, became brownish yellow and generally deposited a flocculent brownish black precipitate of organic matter. Moreover, in some cases, particularly with the livers of dogs, cats and calves, the cuprous oxide, in determining total carbohydrates, would remain dissolved to a great extent, thus interfering with the accuracy of the volumetric determination, sometimes to the extent of even 1–2 c. c. of the sugar solution. In our experiments the acid solution was usually yellow or yellowish brown, and invariably at the end of the 17 hours contained the flocculent precipitate described by Delprat. By nearly neutralizing the solution, the amount of this precipitate was considerably increased and on then filtering the fluid, after having made it up to a volume of 200 c. c., considerable organic matter was removed. This, we found, had a decided influence on the accuracy of the determination, since alkaline solutions of this neutralization precipitate appeared to decidedly retard separation of the cuprous oxide. By paying attention to this point, we had no difficulty in obtaining fairly concordant results, by the use of the Allihn gravimetric method.

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* Pflüger's Archiv, vol. xxviii, p. 121.

† Jahresbericht fur Thierchemie, 1881, p. 323–324.

Experiment I.

A large sized rabbit was killed, the blood collected and defibrinated, the liver quickly removed and finely chopped. Two portions of 40 grams each were weighed out and treated as follows:

A.	*B*
40 grams liver.	40 grams liver.
50 c. c. of a 10 % solution of peptone.	95 c. c. of water.
25 c. c. of blood.	
20 c. c. of water.	

These were placed in flasks, and warmed at 40° C. for two hours. The liver was in contact with the peptone 40 minutes after the death of the animal. A continuous current of air was kept passing through *A.* Following are the analytical results:

Glycogen; total volume of the resultant sugar solution 200 c. c.
Sugar: total volume of the solution 100 c. c.
Total carbohydrates; volume of the resultant solution 200 c. c.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amt.*	Per cent.
		Glycogen A.			
25 c. c.	0 2345 gram.	0·1209 gram.	0·1088 gram.	0·8704 gram	5·44
25	0·2360	0·1217	0·1095	0 8760	5·47
		Glycogen B.			
25 c. c.	0·2675 gram	0·1386 gram.	0·1247 gram.	0·9976 gram.	6·23
25	0·2659	0·1377	0 1239	0·9912	6·19
		Sugar A.			
25 c. c.	0·2260 gram.	0·1164 gram.	-----	0·4656 gram.	2·91
25	0·2255	0·1163	-----	0·4652	2·90

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* Total amount of glycogen, dextrose or carbohydrates calculated as dextrose, contained in the above volume (100 or 200 c. c.) and representing, therefore, the amount contained in two-fifths of the 40 grams of liver.

Sugar B.

Volume used.	Weight Cu.	Equivalent in dextrose.	Total amt.	Per cent.
25 c. c.	0·2135 gram.	0·1097 gram.	0·4388 gram.	2·74

Total Carbohydrates A.

Volume used.	Weight Cu.	Equivalent in dextrose.	Total amt.	Per cent.
12·5 c. c.	0·2160 gram.	0·1111 gram.	1·7776 grams.	11·10
.20	0·3367	0·1768	1·7680	11·05

Total Carbohydrates B.

Volume used.	Weight Cu.	Equivalent in dextrose.	Total amt.	Per cent.
25 c. c.	0·4030 gram.	0 2146 gram.	1·7168 grams.	10·73
25	0 4042	0·2153	1·7224	10·76

The following table shows the average percentage results:

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones and blood (A),	5·46 %	2·91 %	11·08 %
40	Without peptones and blood (B),	6·21	2·74	10·75
		—0·75	+0·17	+0·33

From this it is seen that while in the presence of peptone and blood there is a slight increase of both total carbohydrates and sugar, there is also a more than corresponding decrease in the percentage of glycogen.

Experiment II.

Liver of a rabbit, removed directly after death and treated in the same manner as in Experiment I.

A.	*B.*
40 grams sampled liver.	40 grams sampled liver.
50 c. c. of a 10 per cent. peptone solution.	145 c. c. of water.
25 grams blood.	
70 c. c. of water.	

Warmed 2 hours at 40° C., with a current of air passing through *A.*

Glycogen A.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·3170 gram.	0·1659 gram.	0·1493 gram.	1·1944 grams.	7 46

Glycogen B.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·3420 gram.	0·1798 gram.	0·1618 gram.	1·2944 grams.	8·09

Sugar A.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·2520 gram.	0·1303 gram.	-----	0·5212 gram.	3·26

Sugar B.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
10 c. c.	0·0865 gram.	0·0441 gram.	-----	0·4410 gram.	2·75

Total carbohydrates A.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
10 c. c.	0·2205 gram.	0·1134 gram.	-----	2·2680 grams.	14·15

Total carbohydrates B.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
10 c. c.	0·2110 gram.	0·1084 gram.	-----	2·1680 grams.	13·55

Amount of liver taken.	Method of treatment	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones and blood (A),	7·16 %	3·26 %	14·15 %
40	Without peptones and blood (B),	8·09	2·75	13·55
		−0·63	+0·51	+0·60

Experiment III.

A small rabbit, treated in the same manner as the preceding:

A.	*B.*
25 grams sampled liver.	25 grams sampled liver.
50 c. c. of a 10 per cent. peptone solution.	125 c. c. of water.
50 grams of blood.	
25 c. c. of water.	

Warmed for 2 hours at 40° C., with a constant current of air passing through *A*.

Volume used.	Weight Cu	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
		Glycogen A.			
25 c. c.	0·0435 gram.	0·0226 gram.	0·0203 gram.	0·1624 gram.	1·62
25	0·0455	0·0236	0·0212	0·1696	1·69
		Glycogen B.			
25 c. c.	0·0425 gram.	0·0221 gram.	0·0198 gram.	0·1584 gram.	1·58
25	0·0405	0·0211	0·0189	0·1512	1·51
		Sugar B.[*]			
25 c. c.	0·1413 gram.	0·0719 gram.	-----	0·2876 gram.	2·87
25	0·1400	0·0713	-----	0·2852	2·85
		Total carbohydrates A.			
25 c. c.	0·1560 gram.	0·0796 gram.	-----	0·6368 gram.	6·36
25	0·1585	0·0809	-----	0·6472	6·47
		Total carbohydrates B.			
25 c. c.	0·1445 gram.	0·0736 gram.	-----	0·5888 gram.	5·88
25	0·1427	0·0726	-----	0·5808	5·80

Following are the average percentage results:

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates.
25 grams.	With peptones and blood (A),	1·65 %	----	6·42 %
25	Without peptones and blood (B),	1·54	2·86 %	5·84
		+0·11		+0·58

In this experiment there is the same slight increase of total carbohydrates in the presence of peptone noticed in the two preceding experiments. The increase, however, is not great, and it suggests at once the question, whether the differences, although constant, are beyond the ordinary limits of error. This question we have endeavored to answer in the next experiment.

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[*] Sugar *A* was lost.

Experiment IV.

A rabbit's liver removed from the body immediately after death, was prepared in the usual manner. Two mixtures, exactly alike, were then made as follows:

A.	*B.*
25 grams of liver.	25 grams of liver.
100 c. c. of water.	100 c. c. of water.

These were in the bath 23 minutes after the death of the animal and were warmed at 40° C. for 2 hours. The two portions were then extracted and analyzed as in the preceding experiments; the object being to see how great a variation would be obtained by this like treatment of the two portions of sampled liver. Following are the results:

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent
		Glycogen A.			
25 c. c.	0·1575 gram.	0·0802 gram.	0·0721 gram.	0·5768 gram.	5·76
		Glycogen B.			
25 c. c.	0·1585 gram	0·0809 gram.	0·0728 gram.	0·5824 gram.	5·82
		Sugar A.			
10 c. c.	0·0433 gram.	0·0225 gram.	-----	0·2250 gram.	2·25
		Sugar B.			
10 c. c.	0·0430 gram.	0·0224 gram.	-----	0·2240 gram.	2·24
		Total carbohydrates A.			
25 c. c.	0·2340 gram.	0·1207 gram.	-----	0·9656 gram	9·65
		Total carbohydrates B.			
25 c. c.	0·2335 gram.	0·1203 gram.	-----	0·9624 gram.	9·62

Percentage results.

	Glycogen.	Sugar.	Total carbohydrates.
A.	5·76 per cent.	2·25 per cent.	9·65 per cent.
B.	5·82	2·24	9·62
	−0·06	+0·01	+0·03

These results plainly show that when the conditions of the experiment are exactly the same, the average variation in results will be considerably less than 0·1 per cent. Consequently variations greater than this must have their origin in something other than the ordinary errors of analysis. Hence, in the three preceding experiments we have to account for an average increase of about 0·5 per cent. in total carbohydrates in those cases where peptone and blood are both present.

A comparison of Seegen's results* show that while an aqueous solution of peptone alone in contact with fresh liver increases somewhat the percentage of both sugar and total carbohydrates, the addition of blood, kept arterial by the passage of a current of air through the fluid, appears to still further increase the percentage of sugar and carbohydrates. Seegen, moreover, shows by a blank experiment, that blood alone in contact with the liver has no more influence on the formation of carbohydrates than distilled water.

Experiment V.

This experiment was tried mainly to see what influence peptones by themselves in the absence of blood, would have on the formation of sugar and total carbohydrates. Two portions of sampled liver from a large rabbit were treated as follows:

A.	*B.*
50 grams liver.	50 grams liver.
50 c. c. of water containing 2 grams of peptones.	50 c. c. of water.

The solution of peptone was poured over the liver just 45 minutes after the death of the animal. The mixtures were placed in a bath at 40° C. for 3 hours, after which they were allowed to stand at the temperature of the room for 21 hours. They were then extracted and analyzed in the usual manner, with the following results:

Volume used	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
		Glycogen A.			
25 c. c.	0·1900 gram.	0·0973 gram.	0·0875 gram.	0·7000 gram.	3·50
25	0·1915	0·0980	0·0882	0·7056	3·52
		Glycogen B.			
25 c. c.	0·1785 gram.	0·0913 gram.	0·0821 gram.	0·6568 gram.	3·28
		Sugar A.			
25 c. c.	0·4085 gram.	0·2177 gram.	.....	0·8708 gram.	4·35
10	0·1735	0·0887	.....	0·8870	4·43
		Sugar B.			
10 c. c.	0·1745 gram.	0·0892 gram.	.....	0·8920 gram.	4·46
		Total carbohydrates A.			
10 c. c.	0·1985 gram.	0·1017 gram.	.....	2·0340 grams.	10·17
		Total carbohydrates B.			
10 c. c.	0·1830 gram.	0·0937 gram.	.....	1·8740 grams.	9·37

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* Pflüger's Archiv, vol. xxv, p. 172; ibid, vol. xxviii, p. 125.

Average percentage results.

Amount of liver taken	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates
50 grams	With peptones (A),	3·51 per cent	4·39 per cent.	10·17 per cent
50	Without peptones (B),	3·28	4·46	9·37
		+0·23	−0·07	+0·80

Experiment VI.

This experiment is practically a repetition of the preceding one, excepting that the temperature throughout the experiment was about 18–20° C. and the length of time 24 hours. This latter point we deem of considerable importance, for as the results show, the same increase in total carbohydrates in the presence of peptone is apparent here, after 24 hours treatment and also in the preceding experiment after 21 hours treatment, as has been observed at the end of 2 to 3 hours in the presence of peptone and blood. For Seegen lays considerable stress upon the fact that in many animals the newly-formed carbohydrates and sugar, supposed to have their origin in the peptones, are after a time decomposed, so that at the end, say of 24 hours, the content of sugar and total carbohydrates fall back to their original amount, that is, the amount found in the control. Seegen further claims that this point speaks strongly in favor of the action of the liver, as such, on the conversion of peptone, and rabbits' liver according to his experiments is no exception to the rule.* The experiment was as follows:

A.	*B*
40 grams fresh liver (rabbit).	40 grams fresh liver
55 c. c. of water containing 2 grams of peptone.	55 c. c. of water.

These two mixtures stood at the temperature of the room for 24 hours, when they were extracted and analyzed with the following results:

Glycogen A.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount	Per cent.
25 c. c.	0·2767 gram.	0·1436 gram.	0·1292 gram.	1·0336 grams	6·46
Glycogen B					
25 c. c.	0·2515 gram.	0·1299 gram.	0·1168 gram.	0·9344 gram.	5·84
Sugar A.					
25 c. c	0·2745 gram.	0·1424 gram.	-----	0·5696 gram.	[†]3·56
25	0·2655	0·1375	-----	0·5500	3·43
Sugar B					
25 c. c	0·3210 gram.	0·1681 gram.	-----	0·6724 gram	4·20
25	0·3260	0·1709	-----	0·6836	4·27
Total carbohydrates A.					
10 c. c.	0·2100 gram.	0·1079 gram.	-----	2·1580 grams.	13·48
Total carbohydrates B.					
10 c. c.	0·2013 gram.	0·1032 gram.	-----	2·0640 grams.	12·90

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* Pflüger's Archiv, vol. xxv, p. 175

[†] In this determination, difficulty was experienced in obtaining a good reduction.

Average percentage results

Amount of liver taken	Method of treatment	Glycogen	Sugar	Total carbohydrates
40 grams	With peptones (A),	6.46 per cent	3.49 per cent	13.48 per cent
40	Without peptones (B),	5.84	4.23	12.90
		+0.62	−0.74	+0.58

Both of these experiments tend to show that the presence of blood has no especial influence on the percentage of total carbohydrates; fully as great an increase is to be noticed in the presence of peptone without blood, as when the latter is present. Evidently then, if the increase in total carbohydrates noticed in all of our experiments is really due to the post-mortem formation of carbohydrate matter from peptone it is quite certain that blood is not at all essential to the reaction, at least in the livers of rabbits.

It is to be noticed, moreover, in the two last experiments that, in the absence of blood, the sugar is not increased in amount in the presence of peptone. On the contrary, the presence of peptone under such conditions appears to diminish the formation of sugar, glycogen being correspondingly increased. In all of the experiments with rabbits, it is apparent from the results, that any increase of sugar in the presence of peptone is in every instance counterbalanced by a corresponding decrease in glycogen. In the last two experiments, the same relationship between the amount of glycogen and sugar is to be noticed, only here the greatest percentage of sugar is to be found in that portion of the liver which was treated without peptones. This would suggest that blood either facilitates in some manner the action of such amylolytic ferment as is present in the liver, or else that it introduces an additional ferment which causes increased amylolytic action. Blood certainly does not contain any substance convertible into sugar by the action of boiling acids, since the increase in total carbohydrates is no greater in the presence of blood than in the presence of peptone alone.

We have therefore tried the following experiment in order to ascertain whether blood by itself, in the absence of peptone, has any influence whatever on the formation of sugar.

Experiment VII.

A.	*B.*
50 grams of sampled liver (rabbit)	50 grams of sampled liver.
27 grams of blood	92 c. c. of water.
65 c. c of water.	

The blood from the same rabbit was poured over the liver 35 minutes after the death of the animal and the two flasks containing the mixtures were then placed at a temperature of 40° C. for 2 hours. A constant current of air was kept passing through the blood during this time. Following are the results obtained:

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
		Glycogen A.			
25 c c.	0 1270 gram.	0·0647 gram.	0 0582 gram.	0·4656 gram.	2·32
		Glycogen B.			
25 c c.	0·1947 gram.	0·0997 gram.	0·0897 gram.	0·7176 gram.	3·58
		Sugar A.			
10 c. c.	0·1635 gram.	0·0845 gram.	-----	0·8450 gram.	4·22
		Sugar B.			
10 c c.	0·1420 gram.	0·0723 gram.	-----	0 7230 gram.	3 61
10	0·1415	0 0720	-----	0·7200	3·60
		Total carbohydrates A.			
20 c. c.	0 3800 gram.	0·2014 gram.	-----	2·0140	10·07
		Total carbohydrates B.			
20 c. c.	0·3785 gram.	0·2005 gram.	-----	2·0050	10·02

Average percentage results.

Amount of liver taken.	Method of treatment.	Glycogen	Sugar.	Total carbohydrates.
50 grams.	With blood (A),	2·32 per cent.	4·21 per cent.	10·07 per cent
50	Without blood (B),	3·58	3·61	10·02
		—1·26	+0 60	+0·05

Total carbohydrates are not at all affected by the presence of blood, but the percentage of sugar is considerably increased, and in accord with the increase of sugar, is to be noticed a decided decrease in the percentage of glycogen. Evidently then the percentage of sugar in the rabbit's liver is increased in the presence of blood, which increase is due wholly to a more vigorous decomposition of glycogen. Moreover, whenever increase of sugar has been observed in our experiments a corresponding decrease in glycogen has as a rule, also been seen. In this respect, therefore, our results agree with those of Boehm and Hoffmann, as also with those of Delprat. Seegen, however, states in a later communication,* that in rabbits, glycogen is more rapidly changed than in the case of dogs, or in other words that the liver-glycogen of rabbits is less resistant to the action of ferments.

In all of the preceding experiments with the livers of rabbits, it is to be noticed that the sum of glycogen, calculated as dextrose, and the sugar, is not at all equal to the figures representing total carbohydrates, being in every instance considerably less than the latter. Thus in Experiment V, the amount of total carbohydrates is in *A* (with peptones) 10·17 per cent., while the sum of sugar and glycogen calculated as dextrose is but 8·29 per cent.; a deficit of 1·88 per cent. In *B*, likewise, where peptones are not present, there is a similar deficit, amounting in this case to 1·27 per cent. It is not to be supposed that such a deficiency could in any manner arise from errors of analysis and the most natural supposition is that the sugar, determined and calculated as dextrose, might be of lower reducing power; or in other words that it might consist of maltose instead of dextrose, or rather, of a mixture of maltose and dextrose or of a soluble dextrin. O. Nasse* has stated that the dead liver contains dextrose, or a sugar whose reducing power is not increased by heating with dilute sulphuric acid. Seegen and Kratschmer† also state that the dead liver contains dextrose, and further, that the liver sugar is *exclusively* dextrose. This opinion is based mainly upon the fact that the fluid obtained from a calf's liver by pressure, yielded by dialysis and subsequent treatment with alcohol, a saccharine body, which on the addition of an alcoholic solution of potash was converted into the known *dextrose-potash* compound. Musculus and V. Mering,‡ however, claim that in addition to dextrose, the dead liver also contains maltose. This sugar they detected twice; once in the dead liver of a dog, 1 hour after death, and again, also in a dog's liver, 5 hours after death. In both cases dextrose was likewise present. Dextrin they were not able to detect with certainty, but they consider that the liver ferment also forms this body, intermediate between glycogen and the sugars. E. Külz§ has also prepared from the dead livers of dogs pure dextrose, but he does not conclude definitely as to the presence of dextrin and maltose. It is to be seen, therefore, that while there is unanimity of opinion regarding the presence of dextrose, there is less certainty regarding the presence of the lower reducing sugar, maltose.

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* Pflüger's Archiv, vol. xxiv, p. 467.

We have therefore, carefully examined the nature of the sugar remaining in the alcoholic filtrate after the precipitation of glycogen, and we find, in almost every instance, that the saccharine body there present, has its reducing power considerably increased by heating for a short time with 2 per cent. sulphuric acid. Moreover, the sum of glycogen calculated as dextrose, and the liver sugar converted wholly into dextrose by boiling with dilute acid, exactly equals the total carbohydrates in those cases where peptones are not present. Thus in Experiment V, *A* and *B*, the final sugar solution amounted in each instance to 100 c. c.; 50 c. c. of these solutions were mixed with sufficient 10 per cent. sulphuric acid to insure a content of 2 per cent., after which the two solutions were boiled for two hours, evaporation being prevented by an inverted Liebig's condenser. On cooling, the solutions were made nearly neutral, concentrated somewhat and finally brought back to a volume of exactly 50 c. c. Following are the analytical results both before and after boiling with the dilute acid.

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* Bemerkungen zur Physiologie der Kohlehydrate Pflüger's Archiv, vol. xiv, p. 473

† Die Natur des Leberzucker. Pflüger's Archiv, vol. xxii, p. 214.

‡ Ueber die Umwandlung von Starke und Glycogen durch Diastas, Speichel, Pancreas und Leberferment. Zeitschrift für physiologische Chemie, vol ii, p. 417.

§ Ueber die Natur des Zuckers in der todtenstarren Leber. Pflüger's Archiv, vol. xxiv, p. 52.

A. With peptones.

	Volume used	Weight Cu	Equivalent in dextrose.	Total amount	Per cent
Before boiling,	10 c. c.	0·1735 gram.	0·0887 gram	0·8870 gram	4·43
After boiling,	10	0·2370	0·1169	1·1690	5·84
			B. Without peptones.		
Before boiling,	10 c. c.	0·1745 gram.	0·0892 gram	0·8920 gram	4·46
After boiling,	10	0·2234	0·1150	1·1500	5·75

Hence, it is evident that the liver sugar, in this instance at least, is not made up entirely of dextrose. Neither is it wholly maltose, for if such were the case, the reducing power before and after boiling with acid, would be in the proportion of 66 : 100, whereas in the above experiment the ratio in *A* is 76·4 : 100 and in *B* 78·1 : 100. Still, it would appear that the lower reducing body is present in the largest quantity.

It was not our purpose to study particularly the nature of the liver sugar, so we have not sought for positive proof of the character of this lower reducing body. That it is not dextrin, or glycogen left unprecipitated by the alcohol, is evident from the fact that the addition of a little pure yeast to the normal sugar solution sets up a fermentation by which the sugars are not only completely decomposed but no amylaceous bodies whatever remain in the fluid; at least none which will yield reducing bodies on boiling with dilute sulphuric acid. Consequently it would appear that the lower reducing body is in all probability maltose. We do not intend to say, however, that dextrin is never present in the liver; on the contrary, we are inclined to agree with Musculus and v. Mering that dextrin is doubtless formed as an antecedent to maltose, but in the two or three fermentation experiments that we have tried, the sugar solution has never contained dextrin. It would of course be more natural in seeking for dextrin to look in the glycogen precipitate. Finally, it is interesting to notice in connection with this same experiment, that in *B* the sum of glycogen calculated as dextrose and the sugar after boiling with sulphuric acid equals the total carbohydrates found; while in *A* where peptones are present, the total carbohydrates more than equal the sum of glycogen and sugar, as is shown by the following figures from Experiment V.

	Sugar not boiled with acid		Sugar boiled with acid.	
	A	*B*	*A.*	*B*
Glycogen calculated as dextrose,	3·90 p. c.	3·64 p. c.	3·90 p. c.	3·64 p. c.
Sugar calculated as dextrose,	4·43	4·46	5·84	5·75
	8·33	8·10	9·74	9·39

Total carbohydrates found in *A*	10·17 per cent.
" " " *B*	9·37

This shows an apparent increase in total carbohydrates of 0·43 per cent. (in A) under the influence of the peptones; this being the difference between the total carbohydrates found, and the sum of glycogen calculated as dextrose and the sugar after boiling with dilute acid. In *B* on the other hand, the total carbohydrates found and the sum of glycogen and sugar differ but 0·02 per cent.

Experiment VIII.

This experiment was tried mainly to verify the preceding statements regarding the liver sugar.

40 grams of liver from a rabbit were finely divided and placed in a flask with 50 c. c. of water. The mixture was then warmed at 38–40° C. for 2 hours, when it was boiled and extracted in the usual manner. The liver was placed in the warm bath just 25 minutes after the death of the rabbit, consequently 2½ hours intervened between the death of the animal and the stopping of ferment action. Following are the results of the analysis:

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
Glycogen					
25 c. c.	0·2990 gram.	0·1560 gram.	0·1404 gram	1·1232 grams.	7·02
Sugar.					
10 c. c.	0·0915 gram.	0·0466 gram.	-----	0·4660 gram.	2·91
Sugar after boiling with dilute H_2SO_4.					
10 c. c.	0·1145 gram	0·0582 gram.	-----	0·5820 gram.	3·63
Total carbohydrates.					
10 c. c.	0·1790 gram.	0·0916 gram.	-----	1·8320 grams.	11·45

Glycogen as dextrose.	7·80 per cent		7·80 per cent
Sugar.	2·91	After boiling with H_2SO_4,	3·63
	10·71		11·43

It is evident that here, as in the preceding experiment, the sum of glycogen calculated as dextrose and the sugar equals the total carbohydrates actually found, only when the sugar has been boiled with dilute sulphuric acid, in which case the agreement is, as before, almost exact. Plainly then, dextrose is not the only sugar formed in the liver, and if, as seems probable from our experiments, the sugar has its origin in the hepatic glycogen, it would be quite in accord with analogy to expect the presence of both maltose and dextrose.

The relative reducing power, before and after boiling with acid, is much the same as in the preceding experiments, viz: 79·9: 100, and indicates the presence of considerable of the body with lower reducing power. This portion of the experiment was duplicated with another 40 grams of liver from the same rabbit under exactly the same conditions as to time and temperature, with results almost identical with those obtained from the preceding portion, thus testifying to the accuracy of the methods.

Sugar solution before boiling with dilute acid.

10 c. c. gave 0·0865 gram Cu=0·0444 gram dextrose=2·78 per cent.

Sugar solution after boiling with dilute acid

10 c. c. gave 0·1080 gram Cu=0 0550 gram dextrose=3 44 per cent.

The relative reducing power before and after boiling with dilute sulphuric acid is in this case 80·0 : 100.

Experiments were now tried, to ascertain the influence of peptone on the formation of carbohydrates in the livers of other animals.

Experiment IX.

A large cat in full digestion, fed mainly on proteid matter, was chloroformed, blood collected from the jugular vein and the liver at once removed. Two mixtures were then prepared as follows:

A.	*B.*
50 grams sampled liver,	50 grams sampled liver.
20 grams blood,	100 c. c of water.
50 c. c. of a 10 per cent. solution of peptone	
40 c. c. of water.	

These were placed in a bath at 38–40° C. 50 minutes after the death of the animal, and were kept there for 2½ hours, with a constant current of air passing through *A* in order to render the blood arterial. At the end of this time further action was prevented by boiling the mixtures, after which they were extracted in the usual manner. Neither of the two extracts contained enough glycogen for estimation. Following are the other results:

Volume used	Weight of Cu	Equivalent in dextrose	Total amount	Per cent
		Sugar A.		
25 c. c.	0·1710 gram.	0·0874 gram.	0·3496 gram	1·74
		After boiling with dilute H_2SO_4.		
25 c. c.	0·1730 gram.	0·0885 gram.	0·3540 gram	1·77
		Sugar B.		
25 c. c.	0·1638 gram.	0·0837 gram.	0·3348 gram.	1·67
		After boiling with dilute H_2SO_4.		
25 c. c.	0·1750 gram	0·0895 gram.	0·3580 gram.	1·79
		Total carbohydrates A.		
25 c. c.	0·1048 gram.	0·0533 gram.	0·4264 gram	2·13
		Total carbohydrates B.		
25 c. c.	0·0930 gram.	0·0474 gram.	0·3792 gram.	1·89

Amount of liver taken.	Method of treatment	Glycogen	Sugar.		Total carbohydrates
			Before boiling.	After boiling	
50 grams	With peptones and blood (A),	0	1·74 %	1·77 %	2·13 %
50	Without peptones and blood (B),	0	1·67	1·79	1·89
			+0·07	−0·02	+0·24

In the presence of peptone, there is to be noticed a slight increase in total carbohydrates, which increase receives confirmation from the fact that in *A*, the total carbohydrates exceed the sugar by 0·36 per cent., while in *B* the difference after boiling the sugar with dilute acid is but 0·1 per cent., probably within the limits of error. It is to be noticed, moreover, that in the presence of peptone, the sugar formed is wholly dextrose, its reducing power not being materially changed by boiling with dilute acid, whereas in *B* there is a slight increase in reducing power on boiling with acid. If the difference is sufficient to warrant any explanation it might be suggested that peptone by its presence* stimulates the ferment which presumably converts maltose into dextrose and thus in *A* complete conversion was effected sooner than in *B*.

Experiment X.

This experiment was like the preceding, with the exception that the cat employed was not in full digestion. The stomach was nearly empty and the small intestines likewise. The sampled liver was in the bath at 40° C., 25 minutes after the death of the animal.

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* Compare Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 343.

A.	*B*
40 grams of liver.	40 grams of liver.
25 grams of blood.	135 c. c. of water.
100 c. c. of a 2 per cent solution of peptone	
10 c. c. of water.	

These mixtures were warmed at 40° C. for 2 hours, with a current of air passing through *A*, after which they were extracted and treated in the usual manner. Glycogen was not found in sufficient quantity for determination in either *A* or *B*. An accident happened to the sugar solutions, so that the absolute amount of sugar could not be estimated, but the relative reducing power of the sugar solution, before and after boiling with dilute sulphuric acid, was accurately determined.

Volume used	Weight of Cu.	Equivalent in dextrose	Total amount	Per cent.
		Total carbohydrates A.		
25 c. c.	0·1070 gram.	0·0545 gram.	0·4360 gram.	2·72
		Total carbohydrates B.		
25 c. c.	0·0865 gram.	0·0441 gram.	0·3528 gram.	2·20
		Sugar A.		
25 c. c.	0·1045 gram.	0·0529 gram.	Before boiling with dilute acid.	
25	0·1479	0·0753	After boiling with acid.	
		Sugar B.		
25 c. c.	0·1453 gram.	0·0740 gram.	Before boiling with dilute acid.	
25	0·1925	0·0986	After boiling with acid.	

In this case, the total carbohydrates show an increase of 0·52 per cent. in the presence of peptone. The sugars, unlike the result in the preceding experiment, show a very great increase in reducing power, on being boiled with dilute sulphuric acid. In *A*, the relative reducing power before and after boiling with the acid, is 70·6 : 100 and in *B* 75·4 : 100. Hence in this experiment it would appear that the lower reducing body, presumably maltose, is present in great excess.

Experiment XI.

In this experiment, a liver was taken from a freshly killed lamb, sampled in the usual manner and then warmed at 40° C., with and without peptone, for 4 hours. The liver was in the bath and mixed with the peptone solution just one hour and 20 minutes after the death of the animal. In this experiment no blood was used.

A.	*B.*
50 grams of liver.	50 grams of liver.
50 c. c. of a 4 per cent solution of peptone.	50 c. c. of water.

Glycogen could not be detected in sufficient quantity for estimation. Following are the other results:

Volume used.	Weight of Cu.	Equivalent in dextrose.	Total amount.	Per cent.
		Sugar A.		
25 c. c.	0·2440 gram.	0·1260 gram.	0·5040 gram.	2·52
		After boiling with dilute H_2SO_4.		
25 c. c.	0·2405 gram.	0·1289 gram.	0·5156 gram.	2·57
		Sugar B.		
25 c. c.	0·2430 gram.	0·1255 gram.	0·5020 gram.	2·51
		After boiling with dilute H_2SO_4.		
25 c. c.	0·2445 gram.	0·1262 gram	0·5048 gram.	2·52
		Total carbohydrates A.		
25 c. c.	0·1185 gram.	0·0603 gram.	0·4824 gram.	2·41
		Total carbohydrates B.		
25 c. c.	0·1210 gram.	0·0616 gram.	0·4928 gram.	2·46

Here there is neither increase in total carbohydrates in the presence of peptone, nor is there any change in the reducing power of the sugar solutions after boiling with dilute acid. Total carbohydrates, moreover, fall a trifle below the percentage of sugar, although the difference is within the limits of error.

Experiment XII.

With a sheep's liver, 24 hours after death.

A.	*B.*
50 grams of sampled liver.	50 grams of sampled liver.
100 c. c. of a 2 per cent. solution of peptone.	100 c. c. of water.

These were warmed at 40° C. for 1½ hours, then extracted and tested. No glycogen was found.

Volume used.	Weight of Cu.	Equivalent in dextrose.	Total amount.	Per cent.
		Sugar A.		
25 c. c.	0·1085 gram.	0·0552 gram.	0·2208 gram.	1·10
		Sugar B.		
25 c. c.	0·0825 gram.	0·0420 gram.	0·1680 gram.	0·84
		Total carbohydrates A.		
25 c. c.	0·0750 gram.	0·0383 gram.	0·3064 gram.	1·53
		Total carbohydrates B.		
25 c. c.	0·0465 gram.	0·0241 gram.	0·1928 gram.	0·96

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones (A).	0	1·10 per cent.	1·53 per cent.
50	Without peptones (B).	0	0·84	0·96
			+0·26	+0·57

Increase in both sugar and total carbohydrates is to be noticed in *A*. The increase in total carbohydrates, moreover, is twice as great as the increase in sugar.

Experiment XIII.

With a calf's liver, from a freshly killed animal, obtained at the slaughter house.

A.	*B.*	*C.*
50 grams of liver.	50 grams of liver.	50 grams of liver.
100 c. c. of water.	100 c. c. of water.	100 c c. of a 2 per cent. solution of peptone.

A was warmed at 40° C. for 1 hour and 20 minutes, after which it was extracted and analyzed; *B* and *C* on the other hand, were heated for 1 hour and 30 minutes at the same temperature, after which they were allowed to stand for 16 hours at about 18–20° C. before being extracted. The special object in view was to ascertain the influence of time on the disappearance of glycogen.

Following are the results obtained :

Volume used.	Weight of Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
		Glycogen A.			
25 c. c.	0·0230 gram.	0·0125 gram.	0·0112 gram.	0·0896 gram.	0·44
		Glycogen B.			
25 c. c.	0·0105 gram.	0·0063 gram.	0 0056 gram.	0·0448 gram.	0·22
		Glycogen C.			
25 c. c.	a mere trace only.				
		Sugar A.			
25 c. c.	0·1900 gram.	0·0973 gram.	-----	0·3892 gram.	1·94
		After boiling with dilute H_2SO_4.			
25 c. c.	0·2100 gram.	0·1079 gram.	-----	0·4316 gram.	2·15
		Sugar B.			
25 c. c.	0·2445 gram.	0·1262 gram.	-----	0·5048 gram.	2·52
		After boiling with dilute H_2SO_4.			
25 c. c.	0·2535 gram.	0·1310 gram.	-----	0·5240 gram.	2·62
		Sugar C.			
25 c. c.	0·2315 gram.	0·1198 gram.	-----	0·4792 gram.	2·39
		After boiling with dilute H_2SO_4.			
25 c. c.	0·2620 gram.	0·1357 gram.	-----	0·5428 gram.	2·71
		Total carbohydrates A.			
25 c. c.	0·1210 gram,	0·0616 gram.	-----	0·4928 gram.	2·46
		Total carbohydrates B.			
25 c. c.	0·1410 gram.	0·0718 gram.	-----	0·5744 gram.	2·87
		Total carbohydrates C.			
25 c. c.	0·1405 gram.	0·0715 gram.	-----	0·5720 gram.	2·86

	A. Without peptones, 1 hour 20 min. at 10 C.	*B.* Without peptones, 1½ hours at 10 C. and 16 h. at 20 C.	*C.* With peptones, 1½ hours at 10 C. and 16 h at 20 C.
Glycogen ....,	0 14 per cent.	0·22 per cent	0
Sugar before boiling with H_2SO_4	1·94	2·52	2 39 per cent.
Sugar after boiling with H_2SO_4..	2·15	2·62	2·71
Total carbohydrates............	2·46	2·87	2·86

A comparison of these results shows no increase whatever in total carbohydrates in the presence of peptone (*B* and *C.*) Somewhat strange is the apparent increase of 0·41 per cent., in the absence of peptone after standing 24 hours. The percentage of glycogen is diminished one half after standing 24 hours, and, as has been noticed several times before, it is still further diminished in the presence of peptone. Consistent with the theory of the formation of liver sugar from the hepatic glycogen, is the fact that in this experiment increase in sugar accompanies decrease in glycogen. This is shown best in the percentages of sugar, after boiling with dilute sulphuric acid. The relative reducing power of the sugar solutions, before and after boiling with dilute acid, is in *A* 90·1 : 100, in *B* 96·4 : 100, and in *C* 88·3 : 100. In other words the liver sugar in *B* after standing 24 hours is composed mainly of dextrose, the lower reducing body present in larger quantity in *A* having been gradually changed by longer contact with the liver tissue. In *C*, however, although the time was the same as in *B*, the presence of peptone, while it appears to increase the decomposition of glycogen and thus the actual amount of sugar formed, tends to prevent apparently the conversion of the lower reducing sugar into dextrose; hence in *C* the percentage of sugar before boiling with acid is less than in *B*, while after treatment with acid it is greater.

Following is a resumé of the various results obtained with peptones.

Experiment I.—Rabbit's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones and blood.	5·46 %	2·91 %	11·08 %
40	Without " "	6·21	2·74	10·75
	2 hours at 40° C.	−0·75	+0·17	+0·33

Experiment II.—Rabbit's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates
40 grams.	With peptones and blood.	7·46 %	3·26 %	14·15 %
40	Without " "	8·09	2·75	13·55
	2 hours at 40° C.	−0·63	+0·51	+0·60

Experiment III.—Rabbit's liver.

Liver taken.	Treatment.	Glycogen.	Sugar	Total carbohydrates.
25 grams.	With peptones and blood.	1·65 %	---	6·42 %
25	Without " "	1·54	2·86 %	5·84
	2 hours at 40° C.	+0·11		+0·58

Experiment V.—Rabbit's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones.	3·51 %	4·39 %	10·17 %
50	Without "	3·28	4·46	9·37
	3 hours at 40° C.	+0·23	−0·07	+0·80

After boiling the sugar with dilute H_2SO_4, 4·39=5·82 % Ratio 76·4:100.
" " " " 4·46=5·75 " 78·1:100.
+0·07

Experiment VI.—Rabbit's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones.	6·46 %	3·49 %	13·48 %
40	Without "	5·84	4·23	12·90
	24 hours at 18-20° C.	+0·62	−0·74	+0·58

Experiment IX.—Cat's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones and blood.	0	1·74 %	2·13 %
50	Without " "	0	1·67	1·89
	2½ hours at 40° C.		+0·07	+0·24

After boiling the sugar with dilute H_2SO_4, 1·74=1·77 % Ratio 98·8:100.
" " " " " 1·67=1·79 " 93·6:100.
−0·02

Experiment X.—Cat's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones and blood.	0	....	2·72 %
40	Without " "	0	....	2·20
	2 hours at 40° C.			+0·52

Ratio before and after boiling with dilute H_2SO_4, 70·6:100, with peptones.
" " " " 75·4:100, without peptones.

Experiment XI.—Lamb's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones.	0	2·52 %	2·41 %
50	Without "	0	2·51	2·46
	4 hours at 40° C.		+0·01	−0·05

After boiling the sugar with dilute H_2SO_4, 2·52=2·57%
" " " " 2·51=2·52

Experiment XII.—Sheep's liver.

Liver taken	Treatment	Glycogen	Sugar	Total carbohydrates.
50 grams.	With peptones.	0	1·10 %	1·53 %
50	Without "	0	0·84	0·96
	1½ hours at 40° C.		+0·26	+0·57

Experiment XIII.—Calf's liver.

Liver taken.	Treatment	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones.	0	2·39 %	2·86 %
50	Without "	0·22 %	2·52	2·87
	1½ hours at 40° C. and 16 hours at 20° C.	−0·22	−0·13	−0·01

After boiling the sugar with dilute H_2SO_4, 2·39=2·71 % Ratio 88·3:100.
" " " " 2·52=2·62 " 96·4:100.
+0·09

These results show throughout no indications whatever of a formation of sugar from peptones; on the contrary the results are wholly in accord with the formation of liver sugar from the hepatic glycogen. At the same time it is apparent in several cases, that the decrease of glycogen is somewhat in excess of the increase in sugar, which fact would agree with the view of Seegen and Kratschmer that the destroyed glycogen is consumed in some other manner than by conversion into sugar. But this is in the liver after death, from which we cannot assume like action during life. There is further, in the results obtained, strong evidence that the liver sugar, as found after death, is a mixture of maltose and dextrose; the former presumably being converted into the latter by further ferment action. As to the total carbohydrates the results certainly accord in a general way with those obtained by Seegen. In nearly every case, the presence of peptone gives rise to an increase in total carbohydrates; the average increase in 8 experiments is, however, but 0·52 per cent. This increase, though small, is certainly too large to be explained by the assumption of analytical errors, and the increase, moreover, is too constant to admit of such an explanation. Futhermore, it is to be noticed in several instances, that in the presence of peptone the total carbohydrates found are greater than the sum of the boiled sugar and glycogen calculated as dextrose, while in the control they are practically equal; a fact which certainly lends favor to the view that the apparent increase of total carbohydrates is a real increase, due in some manner to the presence of peptone. Opposed to this view, however, or rather to the view that the liver possesses the power of changing peptone into carbohydrates is the fact that in our experiments the same increase of carbohydrates is to be noticed after 24 hours' contact with peptone as after a shorter time; in fact in our experiments, time appears to have no noticeable influence on the total carbohydrates whatever, unless the like results in Experiment XIII are taken as confirmation of Seegen's statement that after a time the newly formed sugar is decomposed and thus the content of total carbohydrates falls back to its original amount; but in this experiment there is no evidence that the total carbohydrates ever were larger in amount. While our results therefore corroborate Seegen's and Kratschmer's statements regarding an increase of carbohydrates in the presence of peptone, we cannot consider that the slight increase in question, far less than that recorded in their experiments, is sufficiently pronounced to decide conclusively upon such an important theory. Furthermore, the noticeable lack of increase in sugar in the presence of peptone, excepting such increase as is attended with decrease in glycogen, is an additional reason for not attaching as much importance to the slight increase in total carbohydrates alone, as might otherwise be done. Consequently we must conclude that, in our opinion, the results obtained in these experiments do not warrant the adoption of this theory regarding the origin of the liver sugar, and that without further proof to the contrary we must still adhere to the formation of liver sugar from the hepatic glycogen.

XIV.—GLOBULIN AND GLOBULOSE BODIES.* By W. KÜHNE, Professor of Physiology in the University of Heidelberg, and R. H. CHITTENDEN.

THE following investigation is a continuation of our previous work with the albumose bodies,† and constitutes the commencement of a study of the various primary cleavage products, formed by the action of pepsin from the better characterized and purer albumins. Our work in this direction was at first begun with the albuminous bodies occurring in blood serum, egg albumin, and in fibrin and from the results then obtained we were led to the conviction‡ that an extension of the work to the single albumins was necessary. particularly to serum albumin, globulin and myosin. In the meantime, our first conjecture that hemialbumose might possibly be a mixture, had been established and led finally to the isolation of the albumose bodies of fibrin.§ At this stage of our work, fibrin was the only albuminous material employed because it was the simplest to prepare, was easily digestible, and hitherto had been mostly used in digestion experiments. These practical considerations, to which must be added the fact that fibrin albumose was later found as a commercial article, were joined with the advantage of being able to make use of, and at the same time extend, the discoveries in the chemistry of digestion which have for decades been made with this particular albuminous material. Other advantages for the use of fibrin in this connection cannot well be claimed, since its peculiar position between the genuine and the coagulated albumins, its contamination with globulin and the presence of nuclein and other components of the white blood corpuscles tend to render it perhaps least fitted for use in an exact study of albumin cleavage; and since fibrin is of importance as a food stuff mainly in the form of the commercial so-called peptone, there are many reasons for proceeding to the study of other digestible substances for further results.

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[*] This and the following article were originally published in the Zeitschrift für Biologie, Band xxii. They are republished here to render the series complete in English.

[†] See Amer. Chem. Jour., vol. vi, p. 3.

[‡] Zeitschrift für Biologie, Band xix, p. 159.

[§] Ibid., xx, p. 11.

As we hope to show, however, the results gained in the study of the albumose bodies from fibrin are by no means to be considered as valueless, but on the other hand as quite advantageous in the treatment of the single albumins, particularly as an aid in the separation of the individual albumose bodies. Thus in our study of globulin, now to be described, we were able to use methods already tested.

The new method of precipitating albumose bodies by ammonium sulphate, which has meanwhile been discovered and which is certainly particularly advantageous, has not been used in our study of the globulose bodies, since these experiments were concluded before the introduction of that method. According to observations made by Dr. Neumeister in the Physiological Institute at Heidelberg, ammonium sulphate appears particularly well adapted to the purification of deuteroalbumose, since after complete removal of protoalbumose by sodium chloride and acid, deuteroalbumose alone is precipitated by addition of the ammonium salt. As, however, we had accomplished a separation of the different globulose bodies in another manner, it seemed unnecessary to use, in addition, the new method.

Globulin.

From the list of albuminous bodies which occur in sufficient quantity, and which at the same time admit of isolation without too great difficulty we have chosen for our first study, globulin. The substance was prepared from the serum of ox blood by the method of Hammarsten, in which repeated quantities of fresh serum were treated with an excess of crystallized magnesium sulphate at a temperature of 30° C. The precipitate so obtained was collected on a filter, washed with a saturated solution of the salt, pressed, dissolved in water, and reprecipitated a second time by the addition of magnesium sulphate. The precipitate was then dissolved in water, a little thymol added, and the solution dialyzed in running water in order to remove the magnesium salt. The final solution was then concentrated at 40° C., after which the globulin was precipitated with alcohol, washed with alcohol and finally extracted with ether. The substance so prepared, and which still contained some magnesium sulphate, was employed in the digestion experiments. A portion of the preparation, dried at 110° C. *in vacuo*, gave by analysis the results contained in the following table. The methods of analysis were the same as those employed in our previous work.*

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* Compare Zeitschrift für Biologie, Band xx, p. 11, and Amer. Chem. Jour., vol vi, p. 3.

ANALYSIS OF GLOBULIN.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found			N %	Ash found gram.	Ash %	$BaSO_4$ from the ash. gram.	S of ash. % of substance.	$BaSO_4$ after fusion with KOH + KNO_3 gram.	S %	S after deducting S of ash. %
						c. c.	T. °C	Pressure. mm								
I	0·6810	0·3888	6·74	1·1425	49·37	----	--	----	----	----	----	----	----	- --	----	----
II	0·5122	0·3120	6·76	0·9270	49·35	----	--	----	----	----	----	----	----	----	----	----
III	0·8265	----	----	----	----	94·63	4·6	761·6	14·17	----	----	----	----	----	----	----
IV	0·5773	----	----	----	----	64·85	5·5	773·8	14·08	----	---	- --	----	---	----	----
V	1·4916	----	----	----	----	----	--	---	----	0·0520	3·49	0·1010	0·93	----	----	----
VI	1·1170	----	----	----	----	----	--	----	----	0·0387	3·47	0·0756	0·92	---	---	----
VII	0·8445	----	----	----	----	----	-	----	----	----	----	----	----	0·1543	2·50	1·57
VIII	0·5032	----	----	----	----	----	--	-- -	----	----	----	----	----	0·0919	2·50	1·58

Percentage composition of ash-free Globulin.

							Average.
C	51·15	51·13	----	---	- --	----	*51·14*
H	6·99	7·01	----	----	- --	----	*7·00*
N	----	----	14·68	14·59	----	----	*14·64*
S	----	----	----	----	1·66	1·67	*1·67*
O	----	----	----	----	----	-- -	*25·55*
							100·00

Digestion of Globulin.

The preparation proved to be extremely difficult of digestion; this fact, however, enabled us to remove from the globulin the magnesium sulphate still remaining, since the large quantity of dilute hydrochloric acid added with the small amount of pepsin, failed at first to dissolve any of the albuminous matter. The undigested residue was then finally treated with an artificial gastric juice much richer in pepsin. 250 grams of the powdered globulin were at first warmed with 5 litres of 0·2 per cent. hydrochloric acid for 24 hours, whereby the substance swelled up to double its former bulk, but only a trace was dissolved; barely enough for a filtered portion to give with nitric acid and heat a slight turbidity. By the addition of 200 c. c. of normal gastric juice* to the swollen mass and warming it at 40° C. for 24 hours longer, the albuminous matter was not appreciably changed; a filtered portion, however, became turbid on neutralization and a small amount of albumose could be detected both with sodium chloride and with nitric acid. The entire mass of swollen globulin was then collected on a cloth filter, washed thoroughly with 0·2 per cent. hydrochloric acid and warmed again at 40° C. for six days with 4 litres of a particularly active gastric juice, containing 0·4 per cent. hydrochloric acid and 0·45 per cent. of solid matter. By this last treatment, a large amount of globulin was dissolved. As the digestive mixture would not filter through cloth it had to be neutralized directly with sodium hydroxide, which gave an abundant neutralization precipitate of so-called parapeptone, easily collected, and from which the fluid filtered perfectly clear. In order to obtain more material for study, the neutralization precipitate, together with the unaltered globulin, was treated a second time with 3 litres of the same active gastric juice for several days, by which the amount of globulin was reduced more than half, as shown by repeated neutralization.

The two neutralized, digestive fluids obtained in this manner, were alike in all respects; noticeably so in the remarkable fact that when weakly alkaline, they became turbid at 53° C., which turbidity increased as the solutions were heated to boiling. Furthermore, when made faintly acid, heat produced in both solutions an abundant flocculent precipitate which had the properties of coagulated albumin. This *coagulum from digested globulin* was purified by successive washings with boiling water, alcohol and ether. The following table shows the composition of the substance.

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* See Zeitschrift für Biologie, Band xix, p. 184.

ANALYSIS OF THE COAGULUM FROM DIGESTED GLOBULIN.

No.	Substance used. gram.	H_2O found. gram.	H	CO_2 found gram.	C	N found.			N	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S	Ash found. gram	Ash
			%		%	c. c.	T. °C.	Pressure mm.	%		%		%
I	0·7229	0·4448	6·82	1·3667	51·55	----	---	---	---	-----	---	-----	----
II	0·4744	0·2950	6·90	0·8971	51·56	----	----	----	----	-----	----	----	----
III	0·5634	-----	----	-----	----	75.0	18·8	765·2	15·75	-----	---	-----	----
IV	0·3546	-----	---	-----	----	47·48	19·6	764·2	15·76	-----	----	-----	----
V	0·6072	-----	----	-----	----	- --	----	----	----	0·0753	1·70	-----	----
VI	0·6474	-----	---	-----	----	----	----	----	----	0·0893	1·89	-- --	----
VII	0·7602	--- -	----	-----	----	----	----	----	---	-----	----	0·0073	0·96
VIII	0·6724	-----	----	-----	---	----	----	----	----	-----	----	0·0060	0·89

The ash consisted mainly of Fe_2O_3.

Percentage composition of ash-free substance.

							Average.
C	52·03	52·04	----	----	----	----	*52·03*
H	6·89	6·97	----	---	----	---	*6·93*
N	----	----	15·89	15·90	---	--	*15·89*
S	----	----	----	----	1·71	1·90	*1·80*
O	----	----	- --	----	----	----	*23·35*
							100·00

Globulose Bodies.

After removal of the above mentioned coagulum, the solution remained perfectly clear at all temperatures up to 100° C., even when rendered more strongly acid and also on subsequent neutralization. On the addition of nitric acid, however, to the cold solution, a precipitate was formed which disappeared on the application of heat, reappearing as the mixture became cool.

Crystals of salt alone, produced a heavy precipitate in the solution, while salt and acetic acid gave a still further precipitate, and when these reagents failed to cause any further precipitation, nitric acid or metaphosphoric acid would still give a noticeable turbidity.

In order to separate the globulose bodies from one another, the entire solution was concentrated on a water bath to the consistency of a thin syrup and then rubbed up in a mortar with salt in substance (the fluid being perfectly neutral), complete saturation being insured by long standing with an excess of salt crystals. The precipitate so formed being separated by filtration, the filtrate was partially precipitated by the cautious addition of 30 per cent. acetic acid saturated with salt, whereby a mixture of proto- and deuteroglobulose was separated. After removal of this precipitate the filtrate was finally treated with more of the above acetic acid until nothing further was precipitated.

The various precipitates were then subjected to strong pressure to remove as much of the salt-saturated fluid as possible, then dissolved in water and dialyzed for the complete removal of the salt and to separate heteroglobulose.

Protoglobulose.

This body, precipitable by sodium chloride alone, was purified by saturating the first dialyzed solution again with salt, then dialyzing a second time under repeated changes of reaction, by the alternate addition of acetic acid and sodium carbonate and finally by neutral reaction, until all chlorine was removed from the solution and the admixed heteroglobulose completely separated. The clear filtered fluid was then concentrated, after which the protoglobulose was precipitated with alcohol, washed with alcohol and ether and so obtained as an almost white powder. The substance, so prepared, gave when rubbed up with cold water, a filtrate not quite clear and with a noticeably alkaline reaction. It differed from a solution of protoalbumose from fibrin in one respect, viz: that on boiling in the presence of a small amount of sodium chloride it became quite turbid, the turbidity, however, disappearing completely as the solution became cool, (the opposite of the albumose reaction : compare Zeitschrift fur Biologie, Band xx, p. 45). We think it may be assumed that this single deviation from the reactions of fibrin-protoalbumose is not due to the presence of impurities (heteroglobulose, etc), because no heteroglobulose whatever separated from a portion of the sample, even after dialyzing a week longer. If the solution was made even very slightly acid or alkaline, the turbidity did not then occur on heating. Further, the small content of ash (0·4 per cent.), which consisted only of calcium sulphate with a trace of ferric oxide, testifies to the purity of the preparation and the completeness of the dialysis. The preparation was analyzed with the results shown in the accompanying table.

Deuteroglobulose.

This body is about as difficult to purify from the preceding one as deuteroalbumose from protoalbumose. We succeeded, however, in separating it by rejecting the first portions of the precipitate produced by acetic acid and sodium chloride and using only the last portions precipitated; or after the acetic acid failed to give any further precipitate, by using the small precipitate produced by the moderate addition of alcohol. This last precipitate naturally enclosed considerable sodium chloride, but the deuteroglobulose was obtained perfectly pure after removing the salt by dialysis, since the globulose solution, even when noticeably acid, gave no turbidity whatever on the addition of salt in substance. The quantity, however, was unfortunately too small for analysis. It sufficed only for determining the reactions, which agreed with those of the substance obtained by the later precipitation with acetic acid except in one particular, viz: that the latter preparation in a neutral or slightly alkaline solution showed an extremely slight turbidity on the addition of crystals of sodium chloride. All other reactions were identical and corresponded so completely with those of deuteroalbumose that it is only necessary to call attention to the latter (see Zeitschrift fur Biologie, Band xx, pp. 26–28, or Amer. Chem. Jour., vol. vi, pp. 46–47) and to especially mention the non-precipitation of deuteroglobulose in a solution free from salt, by nitric acid in any quantity and at any temperature.

Since no heteroglobulose whatever separated from the solution of the last acetic acid precipitate during dialysis, even with repeated change of reaction, the substance was therefore prepared for analysis,

ANALYSIS OF PROTOGLOBULOSE.

No.	Substance used. gram.	H_2O found. gram	H	CO_2 found. gram.	C	N found.			N	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S	Ash found gram.	Ash
			%		%	c. c.	T. ° C.	Pressure mm.	%		%		%
I	0·6707	0·4211	6·97	1·2577	51·13	....	....	....	....	....	....	....	....
II	0·4926	0·3070	6·92	0·9300	51·48	....	....	....	....	...	...	....	....
III	0·5828	....	....	....	....	72·3	18·8	763·56	16·02	....	...	....	..
IV	0·4813	....	....	....	....	59·23	19·9	758·14	16·03	....	..	....	....
V	0·7436	....	....	....	...	....	....	....	....	0·1188	2·19	...	...
VI	0·5765	....	....	....	....	...	....	....	....	0·0924	2·20	....	....
VII	0·9137	....	...	....	....	....	....	....	....	....	....	0·0043	0·47
VIII	0·5124	....	....	....	....	....	...	....	...	....	....	0·0025	0·48
IX	0·3350	0·2100	6·96	0·6308	51·34	....	....	....	....	....	....	....	....

Percentage composition of ash-free Protoglobulose.

								Average.
C	51·38	51·73	51·59	....	...	....	...	*51·57*
H	7·00	6·95	6·99	....	...	....	....	*6·98*
N	....	....	....	16·09	16·10	....	....	*16·09*
S	....	....	....	....	....	2·20	2·21	*2·20*
O	..	....	....	...	...	....	....	*23·16*
								100·00

simply by concentration of the solution freed from chlorine, precipitation with alcohol and washing with ether. The ash (1·17 per cent.) consisted only of calcium phosphate and a trace of sulphate.

Heteroglobulose.

This body was obtained from the gummy precipitate which separated, during dialysis, from the solution of the first precipitate thrown down from the neutralized digestive fluid by salt alone. The sticky mass was separated from the sides of the parchment tubes, dissolved in sodium chloride of from 3 to 5 per cent., reprecipitated by saturation of the solution with salt, the precipitate again dissolved in dilute salt solution and the substance finally separated by long continued dialysis in running water. After thorough washing with water, alcohol and ether it appeared as a light, white powder, not unlike heteroalbumose in general behavior and reactions. After each precipitation and treatment with dilute sodium chloride, heteroglobulose left a residue, which like dysalbumose was readily soluble only in dilute acids. From the following analysis it is to be seen that the preparation, in spite of its long continued and repeated dialysis, contained 2·03 per cent. of ash, which consisted mainly of calcium carbonate with a small amount of phosphate and sulphate.

The composition of the three globulose bodies shows the same slight differences as noticed in the case of the various albumose bodies (from fibrin). Unlike the latter, however, the content of carbon in the globulose bodies never falls below 51 per cent., and furthermore it is always higher than that of the globulin from which the globulose was derived. The percentage of nitrogen, which in the albumose bodies was found a little higher than in fibrin, exceeds that of the globulin more yet, in some cases by more than 1 per cent., and the same holds true of the percentage of sulphur. In contrast to the albumose bodies, the percentage composition of the globulose bodies gives no grounds whatever for the assumption that they arise from the digested globulin by simple hydration. It must not be forgotten, however, that the digestion of globulin by gastric juice is a process quite different from that of fibrin digestion and one hitherto much less clearly understood, since besides the globulose bodies there is formed a large quantity of a substance which is separated by boiling and which resembles ordinary coagulated albumin. Something similar, indeed, has been known ever since Brücke's study of fibrin digestion, but it has long been accepted that the coagulum

ANALYSIS OF DEUTEROGLOBULOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	Ash found. gram.	Ash %
I	0·4711	0·2910	6·86	0·8790	50·88	----	----	----	----	----	----	----	----
II	0·3468	0·2149	6·88	0·6481	50·96	----	----	----	----	----	----	----	----
III	0·5408	----	----	----	----	72·94	19·6	756·10	15·73	----	----	----	----
IV	0·5106	----	----	----	----	69·01	19·4	756·10	15·76	----	----	----	----
V	0·4616	----	----	----	----	----	----	----	----	0·0656	1·95	----	----
VI	0·5634	----	----	----	----	----	----	----	----	0·0693	1·69	----	----
VII	0·6472	----	----	----	----	----	----	----	----	0·0893	1·89	----	----
VIII	0·6540	----	----	----	----	----	----	----	----	----	----	0·0076	1·16
IX	0·6447	----	----	----	----	----	----	----	----	----	----	0·0077	1·19

Percentage composition of ash-free Deuteroglobulose.

								Average.
C	51·48	51·56	----	----	----	----	----	*51·52*
H	6·94	6·96	----	----	----	----	----	*6·95*
N	----	----	15·92	15·95	----	----	----	*15·94*
S	----	----	----	----	1·97	1·71	1·91	*1·86*
O	----	----	----	----	----	----	----	*23·73*
								100·00

Analysis of Heteroglobulose.

No.	Substance used gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c c.	N found. T. °C	N found. Pressure mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	Ash found. gram.	Ash %
I	0·5200	0·3187	6·80	0·9745	51·10	----	----	-----	----	-----	----	----	----
II	0·3475	0·2149	6·87	0·6500	51·00	----	----	-----	----	-----	----	-----	---
III	0·3625	-----	----	-----	---	48·44	20·0	764·74	15·73	-----	----	-----	----
IV	0·3438	-----	----	-----	----	46·07	19·6	764·57	15·79	-----	----	-----	--
V	0·5227	-----	----	----	----	----	----	-----	----	0·0822	2·15	----	---
VI	0·6045	----	--	----	----	----	----	-----	----	0·0916	2·08	-----	----
VII	0·5811	----	---	-----	----	----	----	-----	----	-----	----	0·0120	2·06
VIII	0·4897	----	----	-----	----	----	----	-----	----	-----	----	0·0098	2·00

Percentage composition of ash-free Heteroglobulose.

							Average.
C	52.15	52·05	----	----	----	----	*52·10*
H	6·95	7·02	----	----	--	---	*6·98*
N	----	----	16·05	16·11	----	----	*16·08*
S	----	----	----	----	2·20	2·13	*2.16*
O	----	----	----	----	---	----	*22·68*
							100·00

obtained by Brücke on boiling the neutralized digestive fluid, arose from the globulin present in the fibrin employed, which had not been previously washed with salt water. Globulin, moreover, yields this body in much greater quantity, even after several days' exposure to the action of an energetic gastric juice and it was still found abundantly among the products of a second digestion of the first neutralization precipitate.

Comparison of the Analyses.

	Globulin.	Coagulum from digested Globulin.	Proto-globulose.	Deutero-globulose.	Hetero-globulose.		Hemalbumose from urine* (Osteomalachia)	Hetero-albumose from Fibrin.†	Fibrin ‡
C	51·14	52·03	51·57	51·52	52·10	C	52·13	50·88	52·68
H	7·00	6·93	6·98	6·95	6·98	H	6·83	6·89	6·83
N	14·64	15·89	16·09	15·94	16·08	N	16·55	17·08	16·91
S	1·67	1·80	2·20	1·86	2·16	S	(1·09 ?)	1·23	1·10
O	25·55	23·35	23·16	23·73	22·68	O	23·40	23·92	22·48

In this review of the composition of globulin and of the products of its digestion we have included also an analysis of fibrin, of a fibrin-albumose and of hemialbumose from the urine of a person with osteomalachia.

We call attention again to the latter because its surprising correspondence, especially to heteroglobulose, appears to confirm the belief, expressed in our former paper that the difference in the albumose from urine and that from fibrin depends on the formation of the former from an albuminous body, whose digestion, at least as regards the formation of albumose bodies, was then unknown, and for which we had already turned to globulin.

In order to gain further information concerning the cleavage of globulin in the process of digestion, the remaining material was used in the following experiments.

1. Heteroglobulose dissolved in 0·3 per cent. sodium carbonate and warmed at 40° C. for fourteen days with pure trypsin (with the addition of thymol, as usual) remained perfectly clear, even after neutralization, and failed to yield afterwards any body resembling antialbumid. Among the products of the digestion, there was found in addition to an abundance of antipeptone, only a trace of leucin, no tyrosin whatever, while with bromine water the alcoholic extract,

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*Compare Zeitschrift fur Biologie, Band xix, p. 202. †Ibid., Band xx, p. 40.

‡According to Hammarsten.

which had been dissolved in water after driving off the alcohol, became simply a little darker, but not rose-colored or violet. Hence heteroglobulose is to be considered as belonging to the anti group.

2. Protoglobulose, which still contained some heteroglobulose, when treated in the same manner with trypsin, behaved similarly but afforded besides an abundance of leucin, also some tyrosin and an extract which became deep violet on the addition of bromine water. Hence protoglobulose gives evidence of belonging to the hemi group.

Finally, we submitted to the digestive action of trypsin the third and fifth precipitates (so-called parapeptone) which were separated in continually decreasing quantities by neutralization, after renewed, energetic pepsin digestion of the original globulin. Both failed to yield any coagulum during their digestion with 0·3 per cent. sodium carbonate, and after the trypsin had acted for fourteen days, neutralization with acetic acid yielded a heavy precipitate, while considerable antipeptone was found in the solution. Although the digestion of the third neutralization precipitate still afforded a trace of leucin and tyrosin without giving any reaction with bromine, no leucin, tyrosin or a substance colored by bromine water could be obtained from the precipitate separated after the fifth pepsin digestion.

Hence globulin, like fibrin and other albuminous bodies, yields during pepsin digestion at the last only bodies of the anti group, which are peptonized, though slowly, by trypsin, but yield no further cleavage products.

XV.—PEPTONES. BY W. KUHNE AND R. H. CHITTENDEN.

SINCE there has been discovered in neutral ammonium sulphate a means for the complete precipitation of the albumose bodies, we have been induced to take up anew our former investigations on the behavior and composition of peptones. As these latter bodies are not precipitated by the ammonium salt, we had expected to obtain peptones free from the primary cleavage products of albumin and thereby advance another step in our knowledge of the definite products of the proteolytic action both of pepsin and of trypsin. Renewed investigation was demanded by the probability that hitherto pepsin-peptones entirely free from albumose have never been obtained, for such peptones as are to be found in commerce or in the hands of the most careful investigator of gastric digestion can readily be shown to contain albumose by saturating a solution of the preparation with ammonium sulphate. There will result an abundant precipitate of albumose and a surprisingly small residue of non-precipitated peptones or the entire absence of such a residue. Only antipeptone obtained by trypsin digestion will occasionally form an exception, and even then in most cases we cannot but doubt that the peptones so formed are wholly free from albumose.

In order to be certain of the presence of peptones in a digestive fluid, it must be made slightly acid with acetic acid, rubbed up with ammonium sulphate till saturated and then filtered from the excess of salt and the albumose precipitate. If the filtrate is thereupon treated with a large excess of strong sodium hydroxide and then a few drops of very dilute cupric sulphate be added, the appearance of the rosy red color of the biuret reaction will indicate the presence of peptones. If peptones are absent the fluid will be pure blue without a tinge of violet, since the solution can contain no other albuminous body. Even after an apparently energetic pepsin digestion the latter result is not at all rare, and a heavy precipitate by the ammonium salt is so frequently seen, that it is still to be doubted whether there is a pepsin-acid digestion which causes the disappearance of all albumose. On the contrary, the albumose precipitate after a sufficiently long and energetic trypsin digestion is very slight and peptone is to be found abundantly in the solution.

We have endeavored to prepare pure peptones in quantity from the solution saturated with ammonium sulphate. For this purpose the solution was first freed from the greater part of the salt by concentration and crystallization. During this process a small amount of a nitrogenous substance separated, perhaps albumose formed again from peptones when the solution was vigorously boiled and the temperature rose to 110° C. The mother liquor, after suitable dilution, was boiled with hot saturated baryta water until all ammonia was expelled, during which operation the precaution was taken to use no excess of barium hydroxide and thus decompose the peptones. From time to time, therefore, portions were filtered, tested for sulphate, and when this became small in amount the last portions of sulphuric acid were removed by barium carbonate. From the filtrate, which always contained much barium, the latter was entirely removed by dilute sulphuric acid, either immediately or after a previous purification of the barium peptone compound. The peptones were then precipitated with alcohol and occasionally further purified with phosphotungstic acid. Naturally the large amount of ammonium sulphate to be removed formed a correspondingly troublesome quantity of barium sulphate, which could be handled only in large filtering bags, and occasioned a large loss of peptone in spite of a most careful washing of the precipitate with boiling water and the application of pressure. On evaporating the peptone solution, which contained but little salt, no resinous precipitate resembling albumose was to be seen.

1. *Amphopeptone.*

We have designated as amphopeptone the end product of the digestion of albumin by pepsin and acid. The first attempt to obtain this peptone free from albumose and in a quantity in some degree proportionate to our wants, showed us that there was needed not only the most active digestive fluid possible and long exposure to a temperature of 40° C. but also a very large amount of pepsin. Such a quantity of the ferment could be procured, however, only by first dissolving considerable quantities of the mucous membrane of the stomach in acid; quantities which must be taken into consideration, in addition to the fibrin to be digested, since something is formed in the self-digestion of the mucous membrane which necessarily remains mixed with the peptone. It is known that mistakes have already been committed by not distinguishing the products arising from the material of the mucous membrane, from those derived from the digested substance. For example, Hoppe-Seyler's erroneous assertion that pepsin digestions yield leucin and tyrosin, rests wholly upon this circumstance, for since the digestion of the mucous membrane always commences with the disappearance of a mucilaginous substance, the derivatives of the latter must necessarily be expected in the resultant solution. Probably for this reason, artificial gastric juice which has been prepared from mucous membrane and is no longer mucilaginous, gives a precipitate when treated with alcohol which differs much from the precipitates of albumose and peptone in being almost as elastic as rubber and, as a rule, forming when shaken, a single ball in which the pepsin is then ordinarily inclosed. We have not yet examined this substance closely, since in the course of the investigations to be described, another more suitable method for precipitating and isolating the ferment has been discovered. We shall designate this elastic body for convenience, mucin-peptone. This mucin-peptone might possibly conceal the whole amount of peptone expected from the digested fibrin, or remain mixed with the latter in considerable quantity. In spite of this objection, which we at no time lost sight of, we prepared a quantity of fibrin-peptone without attempting to remove or to prevent the mixture in question. The observations made by Dr. Pollitzer* in the Physiological Institute at Heidelberg, on the influence of pepsin-peptone free from albumose on coagulation of the blood, were performed with such amphopeptone, which is not perfectly pure.

Supported by the following analyses of this peptone in our presumption that it was rendered impure by mucin-peptone, we sought a process that would exclude this impurity. This was found almost of itself after we had noticed that ammonium sulphate invariably precipitated from the acid solutions, in addition to albumose, the entire quantity of active pepsin. While, therefore, nothing capable of digestion with acids could in any way be obtained from the filtrates, an exceedingly active juice was formed by dissolving the precipitate in dilute hydrochloric acid. Hereafter, we accordingly prepared the strong pepsin solution, by simply precipitating large quantities of very concentrated gastric juice containing 0·5 per cent. hydrochloric acid with ammonium sulphate and dissolving the resinous precipitate, which did not contain an objectionable quantity of albumose bodies, in fresh dilute acid. By this means the mucin-peptone was gotten rid of, since it could not be precipitated by ammonium sulphate and thus a new method was found for preparing and isolating pepsin, which we shall enter upon at another time.

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* Verhandl. d. Naturhist. med. Verein zu Heidelberg, N. F. III, p. 293.

1. *Amphopeptone prepared with ordinary gastric juice.*

Gastric juice, prepared from 145 grams of isolated mucous membrane from the fundus of pigs' stomachs by two days self-digestion in two litres of 0·4 per cent. hydrochloric acid, was added to 585 grams of well washed and boiled fibrin, previously swollen in four litres of acid of the same strength and the whole warmed for two days more at 40° C. The thin fluid-like mixture so obtained, was neutralized with sodium hydroxide and then filtered from the undissolved residue of the mucous membranes (nuclei of the gland cells) and the slight neutralization precipitate. After being made slightly acid with acetic acid, the fluid was heated to boiling, evaporated to two litres, then saturated with neutral ammonium sulphate, separated from the slight coagulum and precipitated albumose, again concentrated to one litre and freed from a large portion of the ammonium sulphate by crystallization at 0° C. In order to still further separate the salt, the solution was treated with one litre of absolute alcohol, again placed in the cold. and finally strained through linen to remove the fine powdery salt, which was wholly free from precipitated peptone. After having been freed from alcohol, by vigorous boiling and concentration to the consistency of syrup and from much salt by crystallization, the thick fluid was filtered by suction, boiled after much dilution with a large amount of barium carbonate until the odor of ammonia had vanished. From the solution, separated from the barium sulphate and again much concentrated, alcohol precipitated the peptone as a barium compound which could be freed from salts (especially sodium chloride) by repeated precipitation and boiling with alcohol. Finally the barium-peptone was decomposed as much as possible with dilute sulphuric acid. As was seen later from the concentrated peptone solution, there remained dissolved a trace of sulphuric acid, but only enough to make the fluid assume a slight opalescence after boiling with barium chloride and hydrochloric acid. An attempt was made to purify the isolated peptone by evaporating, precipitating with alcohol, dissolving in water and reprecipitating with alcohol. This did not succeed well, as shown later by the high percentage of ash. By drying first on a water bath, then in an air bath at 105° C. with frequent stirring, which destroyed the firm resinous surface, the peptone gradually became solid, and changed to a puffed up mass. The resulting product could be ground, when cold, to a light, very hygroscopic powder and weighed in this condition 25 grams.

In an attempt to dry the substance for analysis, during which the temperature was allowed to rise to 110° C. it was found impossible to obtain a constant weight, perhaps on account of decomposition setting in, as suggested by an unpleasant odor which had begun to develop while on the water bath. Portions of 8–10 grams lost daily 0·03–0·04 gram. The analyses were accordingly made only after drying many days. Portions purified with alcohol, dissolved in boiling water with addition of hydrochloric acid, gave no reaction to be distinguished when heated with barium chloride.

Carbon, hydrogen and nitrogen were determined as before, the sulphur by a method already used by us to some extent,* viz: by fusion with potassium hydroxide and potassium nitrate according to the method distinguished by Hammarsten as 1*a*.†

The results of the analysis (Amphopeptone A), shown by the following table, were hardly satisfactory and the low percentage of carbon, particularly, was quite a surprise to us, hence we proceeded at once to the previously mentioned preparation of a peptone, which would probably be rendered less impure by derivatives of the mucous membrane and which would, moreover, be easier to purify further.

2. *Amphopeptone prepared with purified pepsin.*

Preparation of the pepsin.—1220 grams of isolated mucous membrane from the fundus of ten pigs' stomachs were warmed at 40° C. with seven litres of 0·5 per cent. hydrochloric acid for six days. The mixture was then saturated directly with ammonium sulphate, by which a resinous precipitate, with large, sticky lumps was formed, easily collected on a cloth filter. After pressing out the salt solution as much as possible and washing with water, the gummy mass was dissolved in five litres of 0·4 per cent. hydrochloric acid and warmed again at 40° C. for a few days. Then for the first time it was filtered through paper. As preliminary experiments had shown that gastric juice which contains small quantities of ammonium sulphate molds easily, the second digestion and the following fibrin digestion were carried on in the presence of 0·25 per cent. of thymol, which wholly prevented the formation of mold. The mass submitted for the second time to self-digestion, gave now with the ammonium salt a much smaller precipitate, which contained only a very

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* Compare our earlier papers. Zeitschrift fur Biologie, vols. xix and xx.

† Zeitschrift für physiol. Chem., vol. ix, p. 288.

AMPHOPEPTONE (A).

No.	Substance used. gram	H_2O found gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ found. gram.	S %	S after deducting S of ash %	$BaSO_4$ from the ash. gram.	S from ash %
I	0·5615	0·3020	6·05	0·8406	40·82	----	--	----	----	----	----	-----	----	----	-----	----
II	0·4269	0·2267	5·90	0·6420	41·01	----	--	----	----	----	----	-----	----	----	-----	----
III	0·5601	0·3005	5·96	0·8410	40·95	----	--	----	----	----	----	-----	----	----	-----	----
IV	0·5632	-----	----	-----	----	70·0	4·2	757·1	15·34	----	----	-----	----	----	-----	----
V	0·4042	-----	----	-----	----	50·2	4·6	764·2	15·42	-----	----	-----	----	----	-----	----
VI	0·5174	-----	----	-----	----	----	--	----	----	0·0426	8·23	-----	----	----	-----	----
VII	0·5625	-----	----	-----	----	----	--	----	----	0·0450	8·00	-----	----	----	-----	----
VIII	0·4930	-----	----	-----	----	----	--	----	----	-----	----	0·0295	0·82	0·59	-----	----
IX	9·7954	-----	----	-----	----	----	--	----	----	-----	----	0·0550	0·94	0.71	-----	----
X	1·0799	-----	----	-----	----	----	--	----	----	-----	----	-----	----	----	0·0183	0·23

Percentage composition of the ash-free substance.

								Average.
C	44·42	44·62	44·55	----	----	----	----	*44·53*
H	6·58	6·42	6·48	----	----	----	----	*6·49*
N	----	----	----	16·69	16·78	----	----	*16·73*
S	----	----	----	----	----	0·66	0·78	*0·72*
O	----	----	----	----	----	----	----	*31·53*
								100·00

small amount of albumose bodies, while a portion digested as a test for the third time, gave in the filtrate from the precipitate produced by the ammonium salt, so faint a biuret reaction for peptones that it was plainly evident, that the slight residue of albumins from the mucous membrane now remaining, could be overlooked without danger.

Digestion of the fibrin.—3800 grams of washed but not boiled fibrin were digested with the twice precipitated pepsin, which was dissolved in ten litres of 0·4 per cent. hydrochloric acid. To obtain as little albumose and as much peptone as possible, the mixture was allowed to remain at 37°–40° C. for two weeks. At the end of that time, filtered portions gave only slight precipitations by neutralization, but a heavy precipitate was obtained with ammonium sulphate, with sodium chloride, with sodium chloride and acetic acid, and still further by sodium chloride and nitric acid or metaphosphoric acid. Nevertheless the filtrate saturated with ammonium sulphate contained much peptone.*

Preparation and purification of the peptone.—For this purpose the filtrate was neutralized with sodium hydroxide, filtered through linen, especially for removing the impurities of the fibrin, the filtrate slightly acidified with acetic acid, concentrated to about four litres, precipitated with an excess of ammonium sulphate, filtered and pressed, the solution boiled with barium hydroxide and finally with barium carbonate and a large quantity of water, until ammonia could no longer be detected. The barium sulphate was then removed by filtration through cloth bags which were repeatedly washed and pressed, the solution evaporated to about four litres, the barium-peptone decomposed with a very slight excess of sulphuric acid, the new precipitate of barium sulphate filtered off, the solution concentrated to two litres, the free acid neutralized with ammonia and after cooling, six per cent. English sulphuric acid (previously diluted) was added; then the sulphuric acid-peptone solution was precipitated with a large excess of phosphotungstic acid, the precipitate washed first with six per cent. sulphuric acid, then with a large quantity of water, after which the compound was decomposed with excess of barium hydroxide and the excess completely removed from the filtrate with sulphuric acid. The peptone solution thus obtained had a distinctly acid reaction, and strange to say, contained hydrochloric acid, which was hardly to be expected after the very careful washing which the precipitate had received. The solution was neutralized with ammonia to render the acid harmless on concentration. Then we succeeded in obtaining the evaporated residue free from ammonium chloride by repeated precipitation and boiling with alcohol.

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* Later experiments have shown that pepsin acts much more energetically if the ammonium sulphate is completely removed by dialysis, before each new solution and digestion of the pepsin-containing precipitate in hydrochloric acid, and further, that *nearly pure pepsin* becomes wholly inactive by being warmed with dilute hydrochloric acid in the presence of even small quantities of ammonium sulphate

As already mentioned, the method gives rise to much loss and the same holds true of the otherwise excellent precipitation of peptone by phosphotungstic acid according to the method of Hofmeister, for so far as our experience extends, peptones cannot be *completely* precipitated in this manner. There arises in the filtrate, containing excess of phosphotungstic acid, not only additional turbidity and precipitation due to peptone, but considerable quantities of peptone are still found in the liquid, which has perhaps remained clear for months, if treated with barium hydroxide;—a circumstance which we were not able to prevent even by strongly acidifying the solution to be precipitated with phosphotungstic acid, with either sulphuric or hydrochloric acid.

Behavior of the peptone.—This peptone was also difficult to convert into a dry state, although we did succeed ultimately in bringing it to a constant weight as a fine, exceedingly hygroscopic powder, by heating for some time at 105° C. *in vacuo.* The first difficulty was found in commencing the drying, for although we treated the glue-like mass repeatedly with absolute alcohol, then for a long time with ether and finally boiled it again with alcohol, thereby changing it into an almost dry, crumbling condition, we were compelled at last to stop its further direct drying, since at 100° C. the preparation took on the consistency of pitch and formed a bulky foam from which alcohol vapor continually escaped. Therefore the alcohol was first driven out by thorough boiling with water and the latter removed as much as possible at 100° C. This was also a tedious performance for, although the substance no longer foamed up so violently, it did not become dry until after many days of stirring and breaking the covering which continually bubbled up. The same proceeding was repeated, although in a less degree, on transferring the substance to the air bath at 105° C., and only the single portions taken for analysis could be brought to a constant weight without puffing up further at 105° C.

While drying, the unpleasant odor noticed from amphopeptone A was also observed here, although only in a slight degree.

The peptone thus obtained appears (when dried at 105° C.) as a dry, light yellowish powder. It can be preserved in this form only when most tightly stoppered. In the air it soon forms large balls, becomes sticky like pitch and melts to a tough mass which does not become visibly thinner. What is truly surprising is the behavior of the peptone towards water. *A bit of the powder wet with a small drop of water hisses and steams like phosphoric anhydride when moistened, and when this, or the powdered but not absolutely dry preparation which no longer hisses, is dissolved in water, a development of heat is to be noticed.* We have observed the same remarkable peculiarity in anti-peptone to be described later.

Analysis of the preparation (Amphopeptone B) dried at 105° C. *in vacuo*, gave the results tabulated in the accompanying table.

Amphopeptone (*b*).

After these results were obtained, an attempt was made to reduce the ash content of the preparation by repeated precipitation with alcohol, which succeeded so well that the substance when dried *in vacuo* over sulphuric acid, later at 106° C. until of constant weight, contained then 2·15 per cent. of ash instead of 3·25 per cent.

I. 0·5500 gram of this preparation gave 0·3400 gram $H_2O = 6·86$ per cent. H and 0 9566 gram $CO_2 = 47·43$ per cent. C.

II. 0·7121 gram of substance gave 98·4 c. c. N at 16·4° C. and 765·5mm. pressure = 16·49 per cent. N.

III. 0·7839 gram substance gave 0·0169 gram ash = 2·15 per cent. of ash.

Therefore in the ash-free substance (Amphopeptone b) there are—

48·47% C, 7.02% H, 16·86% N.

II. *Antipeptone.*

As with amphopeptone, we have formed in various digestion experiments several preparations of antipeptone by the action of trypsin. These preparations have been studied both after purification with alcohol and after further purification with phosphotungstic acid, but in every case after complete removal of the albumose bodies. We have not attempted to meet the objection that the antipeptone is not formed exclusively from the digested fibrin, but in part from the albumins of the pancreas. This would have necessitated experiments with pure trypsin, which seemed too costly to undertake. Instead of this we have studied a peptone which we shall distinguish

AMPHOPEPTONE (B).

No	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %	$BaSO_4$ found gram	S %	S after deducting S of ash %	$BaSO_4$ from the ash. gram	S from the ash %
						c. c.	T. C.	Pressure mm.								
I	0·8347	0·5320	7·08	1·4379	46·97	----	---	----	----	-----	----	-----	----	----	-----	----
II	0·3511	0·2176	6·88	0·6097	47·35	----	---	----	----	-----	----	-----	----	----	-----	----
III	0·4170	-----	----	-----	47·21	----	---	----	----	-----	----	----	----	----	-----	----
IV	0·4694	-----	----	-----	----	63·56	21.2	758·2	15·74	-----	----	-----	-- -	----	-----	----
V	0·6948	-----	----	-----	----	93·88	21·0	758·8	15·75	-----	----	----	----	----	- ---	----
VI	0·6452	-----	----	-----	----	----	---	----	----	0·0210	3·25	-----	----	----	-----	----
VII	0·6712	----	----	-----	----	----	---	----	----	0·0215	3·20	-----	----	----	-----	----
VIII	0·5032	--- -	----	-----	- --	- --	---	----	---	----	----	0·0360	0·98	0·68	-----	----
IX	0·5936	-----	----	-----	----	---	---	----	----	-----	----	0·0477	1·10	0·80	-----	---
X	1·3164	-----	----	-----	----	----	---	----	----	-----	---	-- --	----	----	0·0288	0·30

Percentage composition of the ash-free substance.

								Average
C	48·54	49·93	48·79	----	----	----	---	*48·75*
H	7·31	7·11	----	- --	----	----	----	*7·21*
N	----	----	----	16·26	16·27	----	----	*16·26*
S	----	----	----	----	----	0·71	0·83	*0·77*
O	----	----	----	----	----	----	----	*27·01*
								100·00

as *gland peptone*, being derived exclusively from the self-digestion of the albuminous bodies of the gland substance, without any addition of fibrin or other albumin.

How superior the action of trypsin is to that of pepsin, is seen not alone in the total decomposition of hemipeptone which is accomplished only by the former, but also in the incomparably more rapid and perfect change of albumose to peptone. Hence the particularly troublesome and tedious treatment of antipeptone with ammonium sulphate may well seem superfluous when there is no albumose present. We must however be perfectly sure, by a preliminary test with that salt, that albumose is absent and its use is unavoidable where impure trypsin is employed in large quantities; that is, where an infusion of the pancreatic gland or the so-called artificial pancreatic juice is used. From the latter, ammonium sulphate precipitates a mixture which contains besides unaltered, highly active trypsin, whose isolation we propose later to study in this way, various other bodies, such as albumose, whose removal is necessary in the preparation of pure peptone.

Antipeptone (C).

Preparation of the pancreatic juice.—100 grams of dried ox pancreas, purified with alcohol and ether, were warmed at 40° C. with 500 c. c. of 0·1 per cent. salicylic acid for 12 hours, and filtered through muslin. The residue was then mixed with 500 c. c. of 0·25 per cent. sodium carbonate, a little thymol added and the mixture again warmed at 40° C. for 12 hours. The acid solution, after it had been neutralized, was brought to the same degree of alkalinity with sodium carbonate, a little thymol added and also warmed at 40° C. for the same length of time. After filtering and pressing the residue of tissue, both filtrates were united. The weight of the undissolved residue, dried at 100° C., amounted as usual to 12 grams. Thus the nuclei of the cells, the collagen and the portion of elastin undigested under these conditions, is equal to 12 per cent. of the dry pancreas, freed from fat.

Digestion of the fibrin.—300 grams of dry fibrin, purified by washing and boiling with water, then with alcohol and finally by extraction with ether were softened with boiling water (when the weight amounted to 970 grams after squeezing with the hands), then warmed at 40° C. with 3 litres of 0·25 per cent. sodium carbonate containing 0·5 per cent. of thymol. To this was added the whole infusion obtained from the 88 grams of self-digested pancreas, after which the mixture was continued at 40° C. for six days. At the end of the first day nearly all of the fibrin had disappeared, although a considerable portion appeared to float on the surface of the fluid. When examined more closely, however, this residue proved to be extremely light, hollow, easily crushed and with a somewhat greasy feeling. A similar residue, the amount of which we did not determine, remained at the end of six days and consisted mainly of antialbumid with much tyrosin.

Preparation of the antipeptone.—The solution resulting from the above digestion was made slightly acid with acetic acid, boiled, passed through a filtering bag, concentrated to 1 litre, and freed from a large amount of leucin and tyrosin by crystallization and filtration. The resultant, brownish-looking syrup was treated with alcohol until peptones began to precipitate, and after the latter had been redissolved by boiling the solution, it was placed aside for crystallization. The filtrate, which now contained only a small amount of amido acids, was freed from alcohol by boiling, diluted with a saturated solution of ammonium sulphate, which had also served for washing out the mass of crystals on the filters, and then completely saturated with the ammonium salt in substance. After separating the slight precipitate so formed, in which some leucin and tyrosin was detected, the greater portion of the ammonium salt was removed from the filtrate by repeated concentration and crystallization, while the remainder was gotten rid of, as before, with barium hydroxide and barium carbonate. Since in this case, precipitation with phosphotungstic acid could not yet be employed, we attempted to purify the peptone as much as possible from other products of digestion (amido acids), first, as a barium compound by repeated precipitation and boiling with alcohol, after which the barium-peptone was exactly decomposed with sulphuric acid and the free peptone purified in a similar manner by repeated precipitation and extraction with alcohol, once or twice in the presence of a little acetic acid. The peptone thus obtained, when dried at 105° C., weighed 120 grams. Assuming that albuminous bodies by complete typsin digestion, split up into 50 per cent. of products arising from the wholly decomposable hemipeptone and 50 per cent. of antipeptone not further changed by typsin, then the amount obtained—120 grams—agrees with this so far as it is possible, with the unavoidable losses which the treatment of large quantities in this manner implies. The 388 grams of dry albumin (300 grams of fibrin and 88 grams of self-digested material from the pancreas) would have had to yield 194 grams of antipeptone if there were no loss. The loss, however, of 74 grams noticed in our experiment is sufficiently explained by the noticeable solubility of peptone in the water contained in alcohol, and by the conversion of a portion of the peptone into antialbumid.

Behavior of the antipeptone.—This peptone was still more difficult to dry than the amphopeptone formed by pepsin digestion, and it could only be accomplished after the removal of all alcohol by thorough boiling with water. As the solution became very concentrated on the water-bath, hydrogen sulphide, as shown by reaction with lead acetate, was given off together with a strong odor of valerianic acid, which was also evolved quite noticeably at 105° C. In order to obtain a constant weight it was necessary to dry the mass at 110° C.

The analysis of the product is shown in the accompanying table.

Antipeptone (D).

The behavior of the preceding preparation while being dried, naturally suggested the suspicion that the substance was either decomposable at 100° C. or less in the air, or else that it contained some decomposable admixture. We therefore attempted a further purification of antipeptone and at the same time a more cautious method of drying.

For this purpose another preparation of antipeptone was made in the following manner.

230 grams of commercial dry pancreas, somewhat less active than that employed in the preceding preparation, were warmed at 40° C. for three hours with 1200 c. c. of 0·1 per cent. salicylic acid, after which the mixture was neutralized with sodium carbonate and to it was added directly 1920 grams of boiled, moist fibrin, 82 grams of dry sodium carbonate and 32 grams of thymol. This mixture was warmed at 40° C. for seven days, at the end of which time the residue of the pancreas, the antialbumid produced, and considerable separated tyrosin, formed a noticeable sediment, which was filtered off and pressed, after the residue had been thoroughly washed with water warmed at 40° C. The filtrate was made slightly acid, heated to boiling, and as this produced only a slight precipitate it was immediately concentrated to about three litres. On cooling, an abundant slate-colored precipitate separated, composed almost entirely of tyrosin. After removing this by filtration, the solution was saturated with ammonium sulphate and the resultant filtrate treated as in the

Antipeptone (C).

No	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ found. gram.	S %	S after deducting S of ash. %	$BaSO_4$ from the ash gram	S from the ash %
I	0·5784	0·3323	6·38	0·9503	44·80	----	----	--	----	----	----	----	--	----		---
II	0·5949	0·3412	6·73	0·9776	44·81	----	----	----	----	--	---	----	----	---		-- -
III	0·5771	----	----	----	----	74·36	5·0	763·0	15·95	----	---	--	--	- --	- -	----
IV	0·4463	----	----	----	-- -	57·45	4·8	762·0	15·93	----	-	-- -	-	---		--
V	0·5256	----	---	----	----	----	----	----	--	0·0278	5·28	- --	--	--		----
VI	0·5092	----	----	---	----	----	----	-- -	----	0·0270	5·32	---	----	----		----
VII	1·0348	----	---	--	----	----	----	----	----	----	----	----	----			0·17
VIII	0·5998	----	----	----	----	----	----	----	----	----	----	0·0368	0·84	0·67	0·0130	--
IX	0·6149	----	----	----	-- -	----	---	----	----	----	---	0·0390	0·87	0·70	----	----

Percentage composition of the ash-free substance.

							Average
C	47·30	47·31	----	----	- --	----	*47·30*
H	6·74	6·72	----	----	- -	- -	*6·73*
N	----	----	16·84	16·82	----	---	*16·83*
S	----	----	----	----	0·71	0·74	*0·73*
O	----	----	----	----	----	-- -	*28·41*
							100·00

previous case; that is, a barium peptone compound was formed, purified with alcohol and this exactly decomposed with sulphuric acid.

In order to separate the free peptone, the solution was concentrated at a gentle heat with the addition of a little ammonia, precipitated and boiled with alcohol, the almost liquid precipitate dissolved in water, the solution acidified with acetic acid, concentrated again, precipitated and extracted hot with alcohol, repeatedly washed and kneaded with ether, kept for a long time under ether and then slowly dissolved in as small an amount of cold water as possible. When filtered, a small residue of tyrosin appeared in the preparation. The new solution was concentrated at a gentle heat, precipitated again with alcohol, the peptone boiled and washed with alcohol, allowed to stand for some time with a large quantity of absolute alcohol, again treated with ether as before, and as it had now become friable, it was immediately dried in vacuo over sulphuric acid. On attempting to dry it at 100° C. the melting mass foamed so much that it had to be dried in the air. When this had been done with frequent stirring on the water bath for several days, the mass was finally dried completely *in vacuo*, first at 100° C., then at 105° C. A few weeks of this drying were needed to bring the substance to a constant weight, and in order to prepare the various quantities for analysis they had to remain *in vacuo* over sulphuric acid and at 105° C. for some time. Probably in consequence of the thorough treatment with ether, the preparation when warmed gave much less odor than the former one.

Composition of Antipeptone (D).

I. 0·5061 gram substance gave 0·2875 gram H_2O = 6·31 per cent. H and 0·7937 gram CO_2 = 42·76 per cent. C.

II. 0·4449 gram substance gave 0·2527 gram H_2O = 6·31 per cent. H and 0·7024 gram CO_2 = 43·05 per cent. C.

III. 0·5010 gram substance gave 0·2870 gram H_2O = 6·36 per cent. H and 0·7885 gram CO_2 = 42·91 per cent. C.

IV. 0·4414 gram substance gave 56·0 c. c. N at 21·0° C. and 759·2 mm. pressure = 14·91 per cent. N.

V. 0·5870 gram substance gave 76·5 c. c. N at 21·6° C. and 758·0 mm. pressure = 15·13 per cent. N.

VI. 0·6930 gram substance gave 0·0694 gram ash = 10·01 per cent.

VII. 0·5017 gram substance gave 0·0504 gram ash = 10·04 per cent.

VIII. The ash from 0·5017 gram substance gave 0·0325 gram $BaSO_4$ = 0·89 per cent. S calculated on the original substance.

On account of the large percentage of ash and the large amount of sulphates, the sulphur of the organic matter was not determined.

Percentage composition of the ash-free substance.

						Average.
C	47·52	47·83	47·69			47·68
H	7·01	7·01	7 07			7·03
N	....			16·57	16 80	16·68

Antipeptone (E).

In order to obtain a still purer preparation and especially to free it from the large percentage of ash, a portion of antipeptone (D) was dissolved in boiling water after the first treatment with ether, then when cold the solution was acidified with sulphuric acid to such an extent that it contained 6 per cent. of acid and precipitated with a large excess of phosphotungstic acid. The precipitate, after the manner already described under amphopeptone (B), was washed thoroughly with dilute sulphuric acid and then with water, finally decomposed with baryta, the barium-peptone precipitated with alcohol, washed, the alcohol driven off by heat, the aqueous solution of the compound exactly decomposed with sulphuric acid, the solution concentrated after the addition of a few drops of ammonia, precipitated with alcohol, dissolved again in water, concentrated with the addition of a little acetic acid, again precipitated with alcohol, and the product so obtained treated thoroughly with alcohol and ether in the same manner as preparation D, and finally dried in the same manner as that. The substance so prepared, was lighter colored than the preceding, not quite so hygroscopic, and in drying gave scarcely any odor.

The analysis of the product is shown in the accompanying table.

Antipeptone (F). (*Gland peptone.*)

This peptone was obtained as a bye product in a preparation of trypsin from 1,000 grams of dry pancreas and was formed wholly from the albuminous bodies of the gland substance, after extraction with alcohol and ether. It is not probable that the peptone contained, in any considerable quantity, any products from the digestion of elastin, since the elastic tissue could have been but little altered under the conditions in which the self digestion of the gland took place during the preparation of the infusion, and furthermore there would have been needed for solution in the latter, a finer subdivision

ANTIPEPTONE (E).

No	Substance used gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %	$BaSO_4$ found. gram.	S %	S after deducting S of ash. %	$BaSO_4$ from the ash. gram	S from the ash %
						c. c.	T. °C	Pressure mm.								
I	0·6561	0·3822	6·47	1·0840	45·05	----	----	----	--	----	----	----	- --	----	- -	----
II	0·6892	0·3989	6·43	1·1300	44·71	----	----	----	----	----	---	----	---	----	----	----
III	0·3911	----	----	----	----	59·68	22·8	756 7	17·61	----	----	---	----	----	----	----
IV	0·7900	----	----	----	----	120·0	21·4	757·7	17·63	----	---	----	----	----	---	----
V	0·6379	----	---	----	----	96·63	21·4	757·6	17·58	----	----	---	----	----	----	--
VI	0·5796	----	----	----	---	----	----	---	----	0·0213	3·67	-- -	-- -	----	----	----
VII	0·6332	----	----	----	----	----	--	----	----	0·0233	3 68	----	----	----	----	----
VIII	0·9190	----	----	----	--	----	--	----	----	---	----	0·0500	0·74	0·63	----	-- -
IX	0·5438	----	----	----	----	----	----	----	----	----	----	0·0800	0·76	0·65	----	-- -
X	1·2128	----	----	----	----	---	--	----	----	----	----	--	----	----	0·0100	0·11

Percentage composition of the ash-free substance.

								Average.
C	46·77	46·42	----	----	----	----	---	*46·59*
H	6·71	6·67	- --	----	----	---	-- -	*6·69*
N	----	----	18·28	18·31	18·25	----	----	*18·28*
S	----	----	--	----	-	0·66	0·68	*0·67*
O	----	----	----	----	----	----	----	*27·77*
								100·00

and long continued action. However this may be, the albumins of the pancreas naturally cannot be classified with the substances ordinarily used in digestion experiments, such for example as fibrin, without further investigation, for although there may be substances in the gland cells like serum-albumin, globulin and myosin, there are also many bodies quite different from these, as, for example, the leucoid precipitated by excess of acetic acid, zymogen and trypsinogen, all of which are decomposed by self-digestion and yield amido acids and peptones. So long as trypsin digestions are not ordinarily conducted with pure trypsin, it is of especial interest to find out the composition of the gland peptones, which, as a rule, have invariably been mixed in greater or less quantity with the antipeptones hitherto investigated.

Preparation.—1,000 grams of dry pancreas were warmed at 40° C. for twelve hours with five litres of 0·1 per cent. salicylic acid and 0·25 per cent. of thymol, filtered through muslin, the residue warmed another twelve hours with two litres of 0·25 per cent. sodium carbonate and 0·5 per cent. of thymol, again filtered and pressed, the two fluids united, brought up to an alkalinity of 0·25 per cent. of sodium carbonate and then warmed at 40° C. for three days.

After filtering through paper, the whole solution was slightly acidified with acetic acid and then saturated with five kilos. of ammonium sulphate, by which means there was precipitated a little albumose and all of the trypsin, the further treatment of which is of no interest here, while the gland peptone remained in solution. It is to be noticed in the separation of this peptone that it was treated exactly like preparation (C), excepting that the second purification with ether could be omitted. The preparation, after drying for some time, left a small residue of tyrosin when dissolved in cold water. It was therefore precipitated from this solution with alcohol, then freed from alcohol by boiling with water, dried directly over a water-bath and finally *in vacuo* at 106° C. until a constant weight was obtained. The analysis of the product is seen in the following table.

Antipeptone (G). (Gland peptone.)

This preparation was obtained from the preceding product by the following process; the solution of the peptone was acidified with 6 per cent. of sulphuric acid, precipitated with a large excess of phosphotungstic acid, the precipitate carefully washed, then decomposed with barium hydroxide, the latter exactly removed with dilute sul-

ANTIPEPTONE (F) (*Glandpeptone*).

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm	N %	Ash found. gram.	Ash %	$BaSO_4$ found. gram.	S %	S after deducting S of ash. %	$BaSO_4$ from the ash. gram.	S from the ash. %
I	0·7511	0·4632	6·79	1·1652	41·97	----	--	---	----	----	----	----	----	----	---	----
II	0·3783	0·2301	6·75	0·5830	42·02	----	-	----	----	----	----	- --	--	----	----	----
III	0·5729	----	----	----	-- --	79·12	18·4	752·0	16·08	----	----	----	-- -	----	----	----
IV	0·4792	----	----	----	----	66·36	18·6	754·0	16·15	----	----	----	---	----	----	----
V	0·6459	----	----	----	----	----	--	----	----	0·0352	5·44	----	----	-- -	----	----
VI	0·6494	----	----	----	----	----	--	----	----	0·0367	5·65	----	----	----	----	----
VII	1·2953	----	----	----	----	----	--	----	----	----	----	----	----	----	0·0128	0·13
VIII	0·6509	----	----	----	----	- -	-	----	----	----	- --	0·0289	0·60	0·47	----	---
IX	0·6269	----	----	----	----	----	--	----	----	----	----	0·0270	0·59	0·46	---	----

Percentage composition of the ash-free substance.

							Average.
C	44·42	44·48	----	----	----	----	*44·45*
H	7·19	7·15	- -	----	----	----	*7·17*
N	----	----	17·02	17·09	---	----	*17·06*
S	---	----	----	----	0·51	0·50	*0·50*
O	----	----	----	----	----	----	*30·82*
							100·00

phuric acid, the solution concentrated, the peptone precipitated and washed with alcohol and finally boiled with alcohol. The partially dry product was then dissolved in cold water, leaving a small amorphous residue which gave no reaction for tyrosin. The solution was then concentrated on the water-bath and again precipitated with alcohol, after which it was dried finally, over sulphuric acid in vacuo and at 106° C. in vacuo, until of constant weight.

The following table shows the results of the analysis.

Antipeptone (H). (Gland peptone.)

This product was prepared and purified in exactly the same manner as the former one (G), but was made from another trypsin preparation, in which a smaller amount of dry pancreas was used. When dissolved for the last time in cold water, some little insoluble matter remained (from which it was freed) which, however gave no reaction for tyrosin.

The following table shows the results of the analysis.

GENERAL PROPERTIES OF THE PEPTONES.

We should have liked to study more accurately the physical behavior of the different samples of peptones, especially their optical properties as determined by specific rotary power. It was easy to show that they were all laevo-rotary, but we have not yet succeeded in making any quantitative determinations of sufficient accuracy to be of value. The decidedly brown color of the solutions prevented the use of a sufficiently long tube, or a solution of the proper concentration, necessary to determine specific rotation.

It would be of still greater importance to investigate the rate of diffusion of peptones, it being more necessary from the fact, that previous observations on the diffusion of the products of digestion can have but little reference to pure peptones, but rather to the albumose bodies so long overlooked. We have, however, not as yet begun these investigations owing to lack of material. All of the peptones obtained by us in the dry state, showed considerable rise of temperature when moistened with, or dissolved in water.

It is worthy of notice, physiologically, that according to observations made in the physiological Institute at Heidelberg, by Dr. Pollitzer of New York, no one of the peptones would hasten or retard coagulation of the blood, either when injected into the veins or added to the shed blood, such action being due wholly to certain of the albumose bodies.

ANTIPEPTONE (G) (*Glandpeptone*).

No.	Substance used. gram.	H_2O found gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ found gram.	S %	S after deducting S of ash %	$BaSO_4$ from the ash. gram	S from the ash. %
I	0·4914	0·3168	7·15	0·7600	42·17	----	--	----	----	----	----	----	----	----	---	----
II	0·6475	0·4141	7·10	0·9998	42·10	----	--	----	- -	- --	----	----	---	----	--	----
III	0·6381	----	----	---	----	94·8	17·6	762·2	17·58	----	---	----	----	---	----	----
IV	0·4756	- --	----	----	---	69·6	17·4	763·8	17·37	----	----	----	---	----	----	----
V	0·6898	---	----	-- -	--	--	--	----	----	0·0132	1·91	----	----	----	----	- -
VI	0·6732	----	----	----	--	----	--	----	- --	0 0132	1·96	----	- --	----	---	----
VII	1·3630	----	----	-- -	----	----	--	----	----	----	-- -	----	----	----	0·0087	0·09
VIII	0·8856	---	----	----	----	----	--	----	----	----	----	0·0249	0·38	0·29	----	----
IX	0·6059	-- -	----	----	----	----	--	----	--	----	----	0·0178	0·40	0·31	---	----

Percentage composition of the ash-free substance.

							Average.
C	42·99	42 93	----	--	----	----	*42·96*
H	7·29	7·24	----	----	----	----	*7·26*
N	----	----	17·70	17·91	----	----	*17·80*
S	---	----	----	---	0·30	0·32	*0·31*
O	- -	----	----	----	----	----	*31·67*
							100·00

ANTIPEPTONE (H) (*Glandpeptone*).

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash. %	$BaSO_4$ found. gram.	S %	S after deducting S of ash. %	$BaSO_4$ from the ash. gram.	S from the ash. %
						c. c.	T. ° C.	Pressure. mm.								
I	0·4107	0·2607	7·05	0·6548	43·47	----	----	----	----	----	----	----	----	----	----	----
II	0·5174	0·3243	6·96	0·8280	43·64	----	----	----	----	----	----	----	----	----	----	----
III	0·5940	----	----	----	----	88·48	18·6	762·8	17·58	----	----	----	----	----	---	----
IV	0·5117	-- -	----	----	----	----	----	----	----	0·0106	2·07	----	----	----	----	----
IVa	0·5117	----	----	----	----	----	----	----	----	----	----	----	----	----	0·0038	0·10
V	0·4969	----	----	----	----	----	----	----	----	----	----	0·0240	0·66	0·56	----	----

Percentage composition of the ash-free substance.

					Average.
C	44·39	44·56	----	----	*44·47*
H	7·20	7·11	----	----	*7·15*
N	---	----	17·94	----	*17·94*
S	----	----	----	0·57	*0·57*
O	----	----	----	----	*29·87*
					100·00

A few observations on the taste of peptones are of interest. While the genuine albumins and the albumose bodies excite practically no sense of taste, the less so the purer they are, it appears as if peptones belong to the most offensively tasting bodies. In order to see what an important change the taste of an albuminous body undergoes on digestion, and at the same time what the taste of peptones is, warm 50 c. c. of fresh milk to 40° C. and then add to it a small fragment of soluble trypsin (prepared from ox pancreas), which excites no taste of itself. The milk at first coagulates, then regains its former appearance by solution of the coagulum, but tastes no better than gall. Nevertheless we believe that the especially objectionable taste proceeds not from peptones, but from certain compounds heretofore only accidentally separated from them. For, among our preparations, which as a whole tasted something like roast meat, as if burnt, but above all nauseatingly bitter and astringent even in a 2 per cent. solution, we found one that in a 10 per cent. solution was free from this disagreeable peculiarity and had a pleasant, sweet taste like meat. It is to be noticed that this was the preparation of antipeptone (F) which had not been purified by phosphotungstic acid. Only by taking a large swallow was there noticed a not unpleasant taste, peculiar to certain cheeses after becoming alkaline.

The most important property for the separation and treatment of peptones is for the present, the lack of precipitation by a great number of reagents, which precipitate more or less perfectly albumins and albumose bodies, especially sodium chloride, either alone, or with the addition of acid. We have repeatedly confirmed the observations made by Wenz that even sodium chloride and acetic acid, sodium chloride and nitric acid or metaphosphoric acid do not completely precipitate the albumose bodies. In whatever proportion these additions may be made, there always remains at the end a solution which gives with alcohol a precipitate of salt, in which albumose is still to be found, or from which albumose may be separated by dialysing and concentrating, according to the method already used. The only perfect precipitant of these substances is ammonium sulphate. It is however an error to attribute to this salt the same action on peptones. Wherever peptones occur, they will always be found in the filtrate from a solution saturated with ammonium sulphate, and we must conclude from some opposed statements that in the experiments on which they are based, albumose bodies instead of peptones were present, since we are certain that by means of our new method, it can be generally shown what an unexpected difference exists between the apparently vigorous action of a poorly prepared gastric juice, or commercial preparation of pepsin and the action of solutions actually rich in pepsin. Even where the fibrin almost instantly disappears, the amount of ferment may still be quite insufficient to produce noticeable traces of peptones. Therefore care must be taken not to conclude immediately from a speedy solution, that digestion has been complete, since this is to be determined only by the disappearance of the primary cleavage products of digestion, that is, the change of albumose into peptones.

It was especially interesting to ascertain whether peptones isolated according to the methods already described, were likewise precipitated by ammonium sulphate or by other reagents that precipitate the albumose bodies, a question which was interesting considering the oft-asserted formation of albumin or albumose from peptones. To our surprise we noticed in the beginning that both antipeptone and amphopeptone after complete purification, under certain circumstances gave rise to a turbidity or even a resinous precipitate, not only with ammonium sulphate, but also when their solutions were saturated with salt or when treated with acetic acid, nitric acid or metaphosphoric acid, just as if albumose had been formed or the albumose not completely removed by the previous treatment. Even if these precipitates concerned only a small part of the material in solution, their appearance would need explanation. So far as we can now determine, the occasion of this behavior is a circumstance concerning which we do not care to decide whether it really depends on the formation of albumose from peptone or not. It is to be observed that if the purification of the peptone by sulphuric acid is conducted incautiously, either on decomposing the barium-peptone or on acidifying before precipitation with phosphotungstic acid, the appearance afterward of albumose is avoided provided the solutions, when warm, are never exposed to an excess of acid. That a resinous precipitate appears, while boiling the solution saturated with ammonium sulphate at 110° C. has already been mentioned, but this cannot cause any impurity of the peptone remaining in solution, any more than the well known precipitation of antialbumid during trypsin digestion can occasion an impurity of the antipeptone.

Since the fact is proved that peptones are not precipitated by ammonium sulphate, these bodies are then characterized more than ever by the property long attributed to them of being rendered turbid by very few reagents and completely precipitated by a still more limited number. A list of the latter reagents includes only tannin and mercuric iodide in potassium iodide, while imperfect precipitants of peptones are phosphotungstic acid or phosphomolybdic acid and picric acid.

The following list shows the further action of various reagents.

Reactions of Peptones free from albumose and purified by phosphotungstic acid.

In 5 per cent. solution, after being made noticeably alkaline with a trace of sodium carbonate.

	Fibrin antipeptone.	Fibrin amphopeptone
Acetic acid and potassium ferrocyanide.	At first perfectly clear, later trace of opalescence.	The same.
Neutral lead acetate.	First drop, 0; more, turbidity	The same, but much weaker.
Basic lead acetate	Turbidity immediately; more, strong turbidity.	The same, but weaker.
Mercuric chloride.	First drop, 0; more, strong turbidity.	Turbidity immediately, growing stronger.
5 per cent. cupric sulphate.	At first clear; more, slight turbidity disappearing with great excess.	Nothing.
5 per cent platinum chloride.	Only excess, strong turbidity.	Nothing
Chromic acid.	Nothing.	Nothing.
Ferric chloride.	A trace gives turbidity vanishing with the least excess.	Nothing.
Glacial acetic acid and conc. sulphuric acid.	Brownish red.	The same
Nitric acid.	The color changing yellow in the cold.	The same.
Boiling with conc. hydrochloric acid.	The color becomes slightly darker	The same
Millon's reaction.	At first a heavy white precipitate; on warming, dirty yellow or reddish.	The same, then beautiful red color.

Without desiring to claim especial value in general for these reactions and for smaller differences between the two peptones, some of them, however, may be more closely examined.

The slight intensification of color by boiling with concentrated hydrochloric acid is striking, for we have not ordinarily been able to obtain it, even with peptone in substance or even on the addition of concentrated sulphuric acid. Likewise, the reaction with sulphuric acid and glacial acetic must be described as almost unsuccessful. As this, however, is nothing other than Pettenkofer's test for bile acids, for which its discoverer has recommended concentrated acetic acid as a substitute for sugar, we have also tried the reaction with sugar, without however obtaining any better result, particularly not the beautiful violet-red which albumin and the albumose bodies give. Finally, the poor result of Millon's reaction with antipeptone, in contrast to the brilliant red obtained with amphopeptone, is also to be remarked. To this reaction we shall return later.

It has already been observed by many investigators that among the products of the digestion of albumin, bodies are not infrequently met with, which give little or no lead sulphide on boiling with sodium hydroxide and lead acetate. This is not at all strange, since the peptones prepared by us show on analysis less than one per cent. of sulphur, in striking contrast to the albumins and albumose bodies, all of which contain much larger percentages. In the gland peptones (F, G, H,) the sulphur amounted to only 0·50, 0·31 and 0·57 per cent. respectively. The peculiarity of peptones in giving up a part of their sulphur when warmed with alkali, apparently stands in no direct connection with the percentage amount of sulphur. Indeed, solutions of the antipeptones G and H, of which the first possessed the lowest percentage of sulphur found, showed no browning with this test, and only a trace of it when solid particles of the peptone were heated with a concentrated solution of alkali containing lead. But the gland peptone (F) not purified by phosphotungstic acid and with only 0·15 per cent. of sulphur became slightly darkened in solution. On the other hand, the purest amphopeptone (B) with 0·77 per cent. of sulphur gave the reaction very faintly, while antipeptone C with 0·73 per cent. of sulphur gave it very plainly. Probably the reaction is not to be attributed to the peptones themselves, but proceeds from contamination with an easily decomposed substance containing sulphur, whose removal still depends on chance.

It may be assumed as completely proved that the rose or violet coloration, which the products of pancreatic digestion assume with bromine or chlorine water, is due to some special body and not to antipeptone. We had previously shown this to be the case with the antipeptone obtained by the action of trypsin from antialbumid, and have now also found in all antipeptones purified with phosphotungstic acid, the absence of all color on addition of bromine water, either in large or small quantity.

Finally, not to overlook the most striking reaction of peptones, we mention further the brilliant so-called biuret reaction which is to be seen, if possible, still more intense in the purer peptones, whatever their origin, than in those formerly used.

Composition of the Peptones.

For the sake of comparison we present in the following table the percentage composition of the various samples of peptones, calculated on the ash-free substance. The ash found, consisting in every case of calcium, a little sodium, potassium, traces of barium and iron, carbonic acid, phosphoric acid and sulphuric acid, is placed at the foot of the columns.

	Ampho- (pepsin) peptone from fibrin.			Anti- (trypsin) peptone.					
	A.	B.	b	C.	D	E.	F.	G.	H.
				Prepared from fibrin			Glandpeptone.		
	Containing mucin-peptone	From purified pepsin and purified with phosphotungstic acid.			Purified more with ether	Purified with phosphotungstic acid		Purified with phosphotungstic acid	
C	44·53	48·75	48·47	47·30	47·68	46·59	44·45	42·96	44·47
H	6·49	7·21	7·02	6·73	7·03	6·69	7·17	7·26	7·15
N	16·73	16·26	16·86	16·83	16·68	18·28	17·06	17·80	17·94
S	0·72	0·77	....	0·73	....	0·67	0·50	0·31	0·57
O	31·53	27·01	....	28·41	....	27·77	30·82	31·67	29·87
Ash	8·11	3·22	2·15	5·25	10·02	3·67	5·51	1·93	2·07

The nature of the substance, which at present affords but little proof that we have to deal with a simple body and not a mixture of chemical bodies, places the greatest restrictions on the use of the above values, and only under such reserve is to be understood what is hereafter added. As regards the analysis of the single substances, we are inclined to believe that only in amphopeptone B, is there a difference in the percentage of carbon greater than the ordinary differences naturally to be expected in amorphous materials so difficult to prepare.

Amphopeptone A, with the lowest percentage of carbon, has already been described as a mixture of fibrin-peptone and mucin-peptone. If we have succeeded in removing the latter, by using purified pepsin, then the figures obtained from *B* and *b* may be considered as representing the first gastric peptone prepared free from albumose. The correspondence between the two, in spite of the fact that by continued purification of *b* we succeeded in reducing the ash by more than 1 per cent., we think may be considered as grounds for this assumption. In opposition therefore to the majority of previous statements, including our own, which as now easily understood referred to mixtures of albumose and peptones, there is to be noticed in pure amphopeptone about 1 per cent. lower content of carbon, about as much higher a percentage of nitrogen and 0·3–0·4 per cent. lower content of sulphur.

With antipeptone, the variation from the previous results was less expected, for we are not inclined to believe that in our former long and thorough typsin digestions, any appreciable quantity of albumose remained and in the more recent ones, the precipitate produced by ammonium sulphate was never abundant if the matter separated by boiling in a slightly acid solution was previously removed. The differences found, however, might be readily explained by the fact that the purification of the peptone had this time been more complete, owing partly doubtless to the formation of the barium compound, and partly also to the precipitation with phosphotungstic acid. The content of carbon is seen to be about 1 per cent. lower, the content of sulphur likewise lower and the percentage of nitrogen decidedly higher, in one case as much as 4 per cent. more than before. The real reason for this difference in composition appears to us to lie in the use of much larger quantities of trypsin, which formerly was only possible by using large quantities of the gland substance, so that the fibrin-antipeptone would naturally be obtained mixed with the gland peptone. It is well for the future that we know the composition of these gland peptones, for they differ essentially, in the lower content of carbon (in one case 42·96 per cent.), from all other peptones hitherto investigated. These bodies might be pronounced troublesome intruders with the same right as the mucin-peptone arising from gastric digestion, although we found in it, aside from the percentage composition, no reaction and no property which would serve to distinguish it from the other peptones.

After these considerations, little stress can be laid on the differences between the composition of pepsin and trypsin peptones. We have found, however, another difference which we will examine more closely.

Cleavage of the peptones.

As already mentioned, Millon's reaction appears very brilliant with amphopeptone, but more or less imperfectly with antipeptone. As the reaction is sure only under certain circumstances we have not neglected to perform it in every possible way, either by using the same concentration of solutions of the two peptones under exactly the same conditions, or by trying the most successful variations of the experiment for antipeptone. Thus it was found that the preparations C, D, E, and F, treated in a suitable way, gave an appreciable reaction, but in no case so that more than a dirty, generally orange red precipitate was obtained. On the contrary, all trials with gland peptone purified with phosphotungstic acid, failed to give more than a simple yellow color. Since Millon's reaction for albumin corresponds with the so-called Hoffmann's test for tyrosin, and since with albumin it probably depends on the separation of tyrosin by boiling with the acid solution of murcuric nitrate, if not by the formation of further decomposition products of tyrosin (hydroparacumaric acids) which likewise redden with the test, it might be presumed that antipeptone, in contrast to the amphopeptone of pepsin digestion, forms no tyrosin by cleavage. So far as the action of trypsin is concerned, this was already known, since the real difference between gastric and pancreatic peptones consists in the fact that only the former, when treated with trypsin, yield tyrosin together with leucin and other decomposition products—in our opinion because they contain hemipeptone capable of further cleavage (together with antipeptone). It was also known to us, however, that antipeptone during cleavage with boiling sulphuric acid yields the amido acids, and among them also tyrosin. Renewed investigations on this subject appeared called for now, since we thought ourselves in possession of much purer preparations of antipeptone.

First, we established the possibility of decomposing with trypsin, amphopeptone entirely free from albumose. A few hours' digestion in a small test tube, of 1 gram of peptone in 10 c. c. of water, containing 0·25 per cent. of sodium carbonate with a little thymol and a fragment of purified pepsin, sufficed for this purpose. By concentrating the neutralized solution and boiling the residue with alcohol, a decided residue was obtained in which balls of leucin and bundles of tyrosin were to be seen under the microscope without further preparation. The residue was also colored a beautiful violet with bromine water. We also sacrificed a large quantity of amphopeptone to the same experiment and obtained the tyrosin pure (free from peptone) and tested it, both by Millon's reaction and Piria's test. Further, a few grams of the same preparation were heated for several days with six times its weight of sulphuric acid (2 : 3 water), and after removing the acid with barium hydroxide, tyrosin and leucin were found among the decomposition products.

Antipeptone was now likewise submitted to the above treatment. The method of conducting this experiment with albuminous bodies which we have used successfully, even with 2–3 grams of substance, is as follows. The substance is placed in a small, strong flask standing on an asbestos plate, and five or six times the weight of sulphuric acid of the above mentioned strength is added and the mixture kept boiling as long as desired. The loss of water by evaporation is prevented by connecting to the neck of the flask a glass tube 1 metre long and 1 centimetre in diameter, so that the lower end cannot be closed by a drop of water. The upper end of the tube is drawn out to a capillary point and as it does not become warm at the top during the boiling of the fluid, all loss of water is prevented.

To be as sure as possible, we have treated the antipeptone in this manner for 48 hours. The contents of the flask were then much diluted with water, filtered from the sticky residue always present, made alkaline with a concentrated solution of barium hydroxide, the barium separated from the filtrate by sulphuric acid, after which the clear fluid was concentrated and allowed to crystallize. It is not advisable to remove the sulphuric acid with barium carbonate, since the latter is unavoidably used in excess, for it is very undesirable to have the barium precipitate unnecessarily increased in bulk, as the cleavage products of albumin are difficult to remove even with hot water. Further, the mixure is made alkaline by barium carbonate and soluble barium compounds always appear, which must be removed by sulphuric acid in every case.

In the strongly concentrated solution finally obtained, in case leucin and tyrosin have really been formed, as with most albuminous bodies, their presence may be readily shown microscopically and after suitable separation of the mother liquor, or if necessary, after re-crystallization from dilute alcohol, their chemical reactions may likewise be obtained. We have succeeded after this manner in showing the presence of leucin always in antipeptone; tyrosin, however, only in a few cases, and even then only after repeated crystallization. Whenever tyrosin occurred it was in exceedingly small quantities. From the antipeptones (gland peptones H and G) with which Millon's test had hitherto failed, tyrosin could not be obtained at all, and that tyrosin was really absent in this case was shown by the final examination of the whole united residues by Hoffman's test, which was absolutely negative, while it appeared plainly with the products of the remaining antipeptones.

Although we do not wish to consider the behavior of the gland peptones as a criterion for antipeptone in general, still this result, united with the small amount of tyrosin obtained from the latter, seemed to call for further investigations concerning the decomposition of those primary cleavage products of albumin related to the antipeptones, especially antialbumid. We began this extension of our work because during our treatment of the subject an article of Maly's* appeared, in which he described a very interesting cleavage and oxidation product, obtained by treating albumin with potassium permanganate, which product possesses the essential properties of the albumins, and yet on further decomposition does not yield tyrosin. It is questionable, therefore, whether this property is not the one directly distinguishing the bodies of the anti-group from the primary cleavage products of the albumins.

A few preliminary experiments were made with samples of antialbumid prepared by the action of boiling dilute sulphuric acid, both on fibrin and Thiry's neutralization precipitate from egg-albumin, also with the antialbumid remaining from the digestion of fibrin with trypsin, and finally with a small neutralization precipitate of so-called parapeptone from an incomplete pepsin digestion of fibrin, which we regarded as antialbumose. After these experiments as a whole, had resulted contrary to our expectations, in that a moderate amount of tyrosin appeared after boiling the substance for a long time with sulphuric acid, we submitted to decomposition a preparation from which we thought we could expect a decisive result. This preparation was an antialbumid from egg albumin, made in one of our former investigations as follows: White of egg freed from membrane, was coagulated by heat in an acid solution, the coagulum filtered, thoroughly washed and then heated for a long time at 100° C. with dilute sulphuric acid, the residue filtered, washed thoroughly with water, dissolved in sodium carbonate, precipitated by neutralization, the precipitate dissolved in 0·2 per cent. hydrochloric acid and the antialbumid freed from all other other albuminous bodies by long continued digestion with pepsin. The antialbumid was then separated from the solution by neutralization, washed, dissolved in 0·5 per cent. sodium carbonate, warmed with trypsin at 40° C., and after the antialbumid had partially separated as a jelly-like mass, it was filtered and washed with water, finally with alcohol and ether. This product could not possibly contain any known albumin, albumose or peptone and undoubtedly formed the purest sample of antialbumid yet prepared.

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* Wiener Acad. Sitzungsber., xci, Abth. 5, February, 1885.

By decomposing this body with sulphuric acid, a residue was finally obtained which to our surprise gave but the slightest reaction for tyrosin with Millon's and Hoffmann's test, with Piria's test no reaction whatever, and in spite of endeavors continued for weeks not a single crystal of tyrosin could be detected. Leucin was found in very small quantity and in addition there were seen large lustrous balls of crystals of some nitrogenous substance, too small in quantity to be identified.

Heidelberg, } December, 1885
New Haven, }

XVI.—On the Dehydration of Glucose in the Stomach and Intestines. By R. H. Chittenden.

In a series of interesting communications[*] "On the physiology of the carbohydrates in the animal system," Dr. F. W. Pavy has brought forward evidence to show that glucose, generally considered as the final product of amylolytic action, can be converted within the animal body into a product of less cupric oxide-reducing power; that there exists particularly in the stomach and intestines of rabbits, a ferment which has a dehydrating action upon glucose or dextrose, transforming it into a body akin to maltose in reducing power.

Hitherto, it has been generally supposed that the transformations which carbohydrates undergo in the animal system are in the nature of gradual hydration changes, in which each step forward toward the final product is attended with the formation of bodies of increased cupric oxide-reducing power. Dr. Pavy's results, however, would tend to show that transformations in the opposite direction do occur and this notably in the stomach and intestines of rabbits.

Dr. Pavy's conclusions concerning this dehydration of glucose in the animal system, are based upon changes in the cupric oxide-reducing power of the carbohydrate, after contact with portions of the stomach and intestines for short periods of time at 48·8° C. It is a well known fact that the reducing power of pure glucose is not affected by boiling with dilute sulphuric acid, while under like treatment, maltose and similar bodies are readily converted into glucose or into a body of like cupric oxide-reducing power.

Dr. Pavy finds, as the result of a large number of experiments, that a solution of glucose or grape sugar, by mere contact with the stomach and intestines of a rabbit at 48·8° C. is changed into a body of less cupric-oxide reducing power, and that by boiling with dilute sulphuric acid this product is carried back again into glucose. Thus, in one experiment, 0·138 gram of glucose in contact with strips of stomach from a rabbit for one hour and a half at 48·8° C. showed, after removal of the dissolved albumin by boiling with sodium sulphate, a reducing power calculated to the entire amount equivalent to only 0·080 gram of glucose; while after boiling with dilute sulphuric acid (the solution containing two per cent. H_2SO_4) the cupric oxide-reducing power was increased to the equivalent of 0·134 gram of glucose, or nearly equal to the amount started with.

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[*] Chemical News, 1884, vol. xlix, pages 128, 140, 155, 162, 172 and 183.

It is to be noticed in the experiment just quoted, that the cupric oxide-reducing power, before and after treatment with sulphuric acid, stand to each other in the proportion of 58 : 100, or in about the relation of maltose (61) to glucose (100). In some experiments, however, the reducing power before boiling with dilute acid, was so low as to warrant the belief that dextrins were also formed. This result is a type of many similar ones obtained by Pavy with the stomach and intestines from various animals and in no instance, in the case of rabbits at least, so far as reported, were negative results obtained.

The discovery of such a dehydrating ferment, hitherto unsuspected, appeared to be a matter of so much importance that experiments have been tried in this laboratory from time to time during the past two years, with the view of confirming in part at least some of Dr. Pavy's results. To our surprise, however, in no case, have we been able to obtain results corresponding to those of Pavy's, although the animals experimented with (rabbits and cats) were taken in various stages of digestion. We therefore record here, some of the results simply in the hope that some light may be thrown upon the cause of this discrepancy; or if, as may be, the ferment is not invariably present, some reason may be found for its constant absence in the tissues of the animals experimented with, and thus light be thrown upon the conditions which control its presence.

The glucose used in the following experiments was a sample of crystallized anhydrous glucose presented to the laboratory by Dr. Arno Behr. The sugar was quite pure, as was ascertained by testing both its reducing power and specific rotary power, and more important still, was not at all affected by boiling with dilute sulphuric acid. Thus 50 c. c. of a one per cent. solution of the glucose, mixed with sufficient 10 per cent. sulphuric acid to have the mixture contain two per cent. of H_2SO_4, was boiled for one and one-half hours, the flask being connected with an inverted Liebig's condenser to prevent concentration. The solution was then neutralized, diluted to 100 c. c. and tested with Fehling's solution according to the method of Allihn. 25 c. c. yielded 0·2414 gram of metallic copper, corresponding to 0·1246 gram of glucose, whereas the 25 c. c. of solution should have contained 0·1250 gram of sugar. Evidently then, the reducing power of the sugar is not affected by treatment with dilute acid.

First experiment.—A rabbit in full digestion was killed, the stomach emptied of its contents and then divided into two longitudinal halves along the curvatures. One-half, after being cleansed, was finely divided and placed in a small beaker with 70 c. c. of water containing 0·200 gram of glucose. An equivalent amount of the small intestine, similarly cleaned and divided, was placed in a second beaker in contact with 70 c. c. of water containing 0·150 gram of glucose. In both cases the entire walls, including *muscularis* and *mucosa*, were taken, since Pavy has indicated that the converting principle is situated not on the surface of the mucous membrane, but in the deeper part. The two mixtures were then placed in a bath and warmed at 48·8° C. for nearly two hours; after which they were boiled, crystals of sodium sulphate being added to aid the removal of the dissolved albumin. The individual filtrates and washings were concentrated and finally brought to a volume of 100 c. c. Of this, 25 c. c. were used to determine the cupric oxide-reducing power of the solution directly, while 50 c. c. of each solution were mixed with sufficient ten per cent. sulphuric acid to insure a content of two per cent. and then boiled for two hours, in connection with an inverted Liebig's condenser to prevent concentration. The acid solutions were then neutralized, concentrated somewhat and finally brought back to a volume of 50 c. c. Following are the analytical results obtained with the two solutions, the reducing power being determined by Allihn's gravimetric method.*

STOMACH.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0746 gram Cu=0·0381 gram dextrose ×4=0·1524 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0728 gram Cu=0·0372 gram dextrose ×4=0·1488 gram dextrose.

INTESTINE.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0512 gram Cu=0·0265 gram dextrose ×4=0·1060 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0520 gram Cu=0·0269 gram dextrose ×4=0·1076 gram dextrose.

Here, there is no evidence whatever that the glucose was affected by its two hours' contact with the stomach and intestine of the rabbit at 48·8° C., the temperature specified by Pavy as that best adapted for the reaction. Certainly the reducing power of the glucose solution is essentially the same before and after treatment with sulphuric acid. Somewhat in accord with Pavy's results, however, is the fact that while 200 milligrams of glucose were introduced into the stomach mixture and 150 milligrams with the intestines, only 152·4 milligrams were recovered from the former and 106 milligrams from the latter, although the residues after heating with sodium sulphate, were repeatedly and thoroughly washed with hot water. Assuming that this loss of sugar in the two cases is really due to change of glucose into lower reducing bodies, the relative reducing power of the sugar before and after contact with the stomach and intestines would be 100 : 76·2 and 100 : 70·6 respectively. But if there had been any such change in reducing power, the treatment with sulphuric acid would certainly have indicated it.

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* Zeitschrift für Analytische Chemie, 22. Jahrgang, p. 448

Second experiment.—A rabbit in full digestion was killed and half of the stomach and a portion of the small intestine were cleaned and finely divided. The stomach tissue was then heated at 48·8° C. for one and one-half hours, with 70 c. c. of water containing 0·200 gram of glucose and the portion of intestine for the same length of time, with a like amount of glucose. Treated then in the same manner as the preceding solutions, the following results were obtained:

STOMACH.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0901 gram Cu = 0·0460 gram dextrose × 4 = 0·1840 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0882 gram Cu = 0·0450 gram dextrose × 4 = 0·1800 gram dextrose.

INTESTINE.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0690 gram Cu = 0·0353 gram dextrose × 4 = 0·1412 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0726 gram Cu = 0·0371 gram dextrose × 4 = 0·1484 gram dextrose.

In this experiment, a larger amount of sugar was recovered in the case of the stomach tissue than in the preceding experiment, but in neither the stomach or intestine is there any evidence of change in the reducing power of the sugar before and after treatment with sulphuric acid. In this connection it is to be remembered, that the only ground for belief in the existence of a dehydrating ferment in the stomach is the change, noticed by Pavy, in the reducing power of the sugar under the above method of treatment. Our method of treatment with sulphuric acid, moreover, both as to the length of time the mixtures were heated and the strength of acid employed, was in accord with the method used by Pavy. In addition, the same method of treatment was applied to a known solution of maltose with satisfactory results, viz: a rapid and complete change into dextrose as attested by the proper proportional increase in reducing power. Furthermore, we are led to infer from Pavy's results that the action of the ferment is to be seen to the best advantage in the rabbit. Thus Pavy states,* that without having made any precise comparative observations, 'I am under the impression that the stomach and the intestine of the rabbit act more energetically than the stomach and intestine of the other animals I have tried. It also appears to me that the stomach acts more energetically than the intestine, and in some instances I have noticed that the effect produced, has stood in relation to the amount of ferment material used." The latter half of this statement would tend to indicate that the main reason for our not recovering all of the glucose is to be found either in a lack of sufficient washing of the tissue residue at the end of the experiment, or else in a slight fermentation by which a portion of the sugar might be decomposed; for as is to be noticed in nearly all of the experiments recorded here, far less sugar is lost in the stomach than in the intestine, whereas if due to change in reducing power from the action of a dehydrating ferment, the greatest loss, Pavy's statement being correct, would be observed by contact with the stomach tissue. On the contrary, our results show greatest loss in the intestine, which if due to mechanical reasons would be naturally explained, since the glairy mass of tissue, even after boiling, affords mechanical obstacles to a thorough extraction. That this is doubtless the true explanation, in part at least, is evidenced by the fact that a portion of the stomach or intestine, previously boiled with water to destroy its vitality, yields results after the same order as those already given, except that the amount of sugar recovered is greater, as would naturally be expected since the tissue being already coagulated would not enclose the sugar so completely. Thus, on warming one-half of a rabbits' stomach, previously divided and boiled with water, with 0·200 gram of glucose for two hours, there was recovered 0·1910 gram of the glucose; while from a portion of the small intestine, likewise boiled and treated with the same amount of glucose, there was recovered only 0·1840 gram of the sugar. Furthermore, fermentation of the sugar would naturally occur more quickly in the intestines than in the more compact stomach tissue. Be that as it may, the reducing power of neither solution was affected by boiling with dilute sulphuric acid.

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* Chemical News, vol. xlix, p 141.

Third experiment.—A rabbit in a condition of hunger was killed, the stomach divided longitudinally along the curvatures, and one-half after being cleaned and finely divided, was placed in contact with 0·200 gram of glucose dissolved in 70 c. c. of water, and warmed at 48·8° C. for one and one-half hours. The mixture was then heated to boiling with the addition of some crystals of sodium sulphate, the tissue and coagulated albumin filtered off and the residue washed with about 300 c. c. of hot water. The fluid was concentrated, brought to a volume of 100 c. c. and then treated as in the preceding experiments. Following are the results obtained:

STOMACH.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0834 gram Cu = 0·0425 gram dextrose × 4 = 0·1700 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0798 gram Cu = 0·0107 gram dextrose × 4 = 0·1628 gram dextrose.

Here, as before, there is no evidence of any change in the character of the glucose; still in spite of the comparatively large volume of wash-fluid used, the sugar was not wholly recovered.

Fourth experiment.—A cat killed in full digestion was employed in this experiment. One-half of the stomach, finely divided, was placed in contact with 0·200 gram of glucose in 75 c. c. of water. A portion of the small intestine was also treated with a like amount of sugar, in the same manner. Both were warmed for three hours at 48·8° C., then treated by the same method as used in the preceding experiments.

STOMACH.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0879 gram Cu = 0·0449 gram dextrose × 4 = 0·1796 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c gave 0·0886 gram Cu = 0·0452 gram dextrose × 4 = 0·1808 gram dextrose.

INTESTINE.

a Before treatment with sulphuric acid

25 c. c. gave 0·0668 gram Cu = 0·0342 gram dextrose × 4 = 0·1368 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0684 gram Cu = 0·0350 gram dextrose × 4 = 0·1400 gram dextrose.

Here again, there is no evidence whatever of any change in the reducing power of the sugar solution.

Pavy has also pointed out that the stomach and intestine of the rabbit, as well as of other animals, have a transformative action on saccharose as well as on dextrose. The transformative energy however of the intestine, is much greater in this case than that of the stomach; which, according to Pavy, accounts for the early discovery by Bernard of the well known action of the intestine on cane sugar. Bernard supposed dextrose to be formed, but Pavy shows that maltose or a body resembling maltose in reducing properties, is the usual product and that glucose or dextrose is formed only in the presence of considerable ferment. The ferment which produces this change, unlike the ferment which acts upon glucose, is situated on the surface of the mucous membrane and thus frequently the contents of the stomach are likewise found to possess transformative power.

Fifth experiment.—A rabbit with stomach partially filled with food was killed, the stomach rinsed with water, then minutely divided and separated into two equal parts. One portion was placed in contact with 0·195 gram of glucose in 75 c. c. of water, while the other portion was mixed with a like amount of pure saccharose, also in 75 c. c. of water. A portion of the small intestine was likewise finely divided and one portion placed in contact with 0·195 gram of glucose in 75 c. c. of water and the other portion with 0·200 gram of saccharose dissolved in 75 c. c. of water. All four mixtures were warmed at 48·8° C. for two hours, then heated to boiling with the addition of sodium sulphate and finally each brought to a volume of 100 c. c. 25 c. c. of the saccharose solution, which had been in contact with the stomach tissue, gave no reduction whatever with Fehling's solution. 25 c. c. of the saccharose-intestine solution, however, gave 0·0726 gram Cu, equivalent to 0·0371 gram dextrose.

With the glucose solutions, the following results were obtained:

STOMACH.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0906 gram Cu =0·0462 gram dextrose ×4=0·1818 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0889 gram Cu =0·0451 gram dextrose ×4=0·1816 gram dextrose.

INTESTINE.

a. Before treatment with sulphuric acid.

25 c. c. gave 0·0712 gram Cu =0·0364 gram dextrose ×4=0·1456 gram dextrose.

b. After treatment with sulphuric acid.

25 c. c. gave 0·0689 gram Cu =0·0353 gram dextrose ×4=0·1412 gram dextrose.

With glucose, the same results are to be observed here as in the preceding experiments; the only variations in reducing power, before and after treatment with sulphuric acid, being such as would come within the ordinary limits of error. In one single case, a transformation of saccharose was noticed when the sugar solution was warmed for two hours with a portion of stomach tissue from a rabbit killed in full digestion. The reduction with Fehling's solution was quite strong.

With glucose, however, many experiments have been tried in addition to those given above, and invariably with the same negative result. The conditions of the experiments, moreover, are in many cases identical with those of Pavy's except in the method of determining reducing power. There seems, therefore, to be no plausible explanation of the results obtained, other than that in the above experiments there was no dehydrating ferment present. Pavy states that the ferment in question, or rather "the active principle concerned in the transformation of glucose is susceptible of being destroyed by the agency of gastric digestion," so that there is the possibility of such destructive action having taken place in the stomach of the animals experimented with. It is further stated, however, that the converting principle is situated in the underlying portion of the mucous membrane, so that destruction could hardly be expected, except perhaps in the slow self-digestion occurring after death. Certainly, the carefully rinsed tissue could not have retained sufficient gastric juice to affect the results. Furthermore, such decomposition would apply only to the stomach mixture and not to the intestines, unless sufficient proteolytic ferment from the pancreatic juice should adhere to the walls of the intestines to exert destructive action; but in the last experiment given, it is to be noticed that the saccharose ferment, which is presumably equally sensitive, showed vigorous action while the glucose was unaffected.

It seems strange, therefore, if such a dehydrating ferment is normally present in the alimentary tract, that we have not been able to obtain some tangible evidence of its presence, either in the stomach or intestines.

Since the above was written, the writer has noticed that M. Ogáta,* experimenting with dogs, has also been unable to confirm Pavy's results, both in the dehydration of glucose and in the inversion of saccharose.

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*See Jahresbericht für Thierchemie, xv, 275.

XVII.—Influence of Uranium Salts on the Amylolytic Action of Saliva and the Proteolytic Action of Pepsin and Trypsin. By R. H. Chittenden and M. T. Hutchinson, Ph.B.

Little is known regarding the physiological, or even toxical action of the uranium salts. In 1825, Gmelin* reported upon the results of some experiments on the toxic action of uranic nitrate, but aside from the work done at that time, little is known regarding the action of uranium. It is our purpose, therefore, to carry out in this Laboratory, as opportunity offers, a series of experiments on the physiological and toxical action of uranium salts, and we have commenced the work by endeavoring to ascertain the influence of the above salts on the amylolytic and proteolytic action of the ferments occurring in the digestive fluids of the body. In this connection we wish to express our obligations to Professor H. Carrington Bolton, of Trinity College, for his kindness in supplying us with an abundance of chemically pure uranium compounds.

1. *Influence on the amylolytic action of saliva.*

The method employed in determining the extent of amylolytic action was much the same as that previously† used by one of us, except that the amounts of reducing substances formed under the different conditions of the experiments, were determined volumetrically by Fehling's solution, instead of by Allihn's gravimetric method. The experiments were made in series, in which one digestion of each series served as a control for comparison.

The volume of each digestive mixture was 100 c. c. and contained 1 gram of perfectly pure potato starch, previously boiled with a portion of the water, 10 c. c. of a diluted neutral saliva and a given percentage of the uranium salt to be experimented with. The mixtures were then warmed at 40° C. for 30 minutes, at the end of which time, further ferment action was stopped by heating the solutions to boiling. The saliva employed in the experiments was human mixed saliva, freshly collected, filtered and made as neutral as possible with 0·2 per cent. hydrochloric acid, then diluted with water in the proportion of 1 : 5. Hence, each digestive mixture contained 2 c. c. of undiluted saliva.

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* Edinb. Med. Surg. Gaz., xxvi., 136.

† Studies from this Laboratory, vol. i, 1884–5, p. 2 and 53.

The amount of reducing substances, which for the sake of convenience are calculated as dextrose, were, as already mentioned, determined volumetrically and from the data so obtained, the percentage of starch converted was likewise calculated.

Uranyl nitrate.

With this salt the following results were obtained :

$UO_2(NO_3)_2 + 6H_2O$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action
0	0·4135 gram.	37·21 per cent.	100·0
0·0001 per cent.	0·4083	36·74	98·7
0·0003	0·3873	34·85	93.6
0·0005	0·3698	33·28	89·4
0·001	0·3612	32·50	87·3
0·003	0·3131	28·17	75·5

The inhibitory action of the uranyl salt is plainly manifest in these results. A second series of experiments, with still larger percentages of uranyl nitrate, show the retarding action still more plainly.

$UO_2(NO_3)_2 + 6H_2O$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·4066 gram.	36·59 per cent.	100·0
0·001 per cent.	0·4000	36·00	98·3
0·002	0·3880	34·92	95·4
0·003	0·3034	27·30	74·6
0·004	0·2545	22·90	62·5
0·005	0·1550	13·99	38·2
0·008	trace.		

The largest percentage of the salt used (0·008 per cent.), is seen to almost entirely prevent the action of the ferment, thus showing how extremely sensitive the salivary ferment is to the action of this salt. Comparing the two series of experiments, it is seen further, that a given percentage of the salt, say 0·001 per cent., is much more active in one case than in the other, indicating that the action of the salt is not constant. This is undoubtedly true to a limited extent. The action of a given percentage of the salt is constant only under *like conditions.* In the above series of experiments, the saliva is different in the two cases, and the real explanation of the difference in action is to be sought for in the amount of proteid matter contained in the saliva. Undoubtedly the retarding action of the uranium salt is checked, in part at least, like that of mercuric chloride,* by the proteid matter of the saliva. Uranium is a well-known precipitant of albuminous matter and hence the larger the amount of albumin and globulin contained in the saliva, the weaker the retarding action of the uranium salt. Previous experiments have shown that saliva varies somewhat from day to day in its content of proteid matter, as well as in the amount of ferment, and although the former difficulty is obviated as much as possible by diluting the saliva, still this point must be borne in mind in making comparisons of the different series of experiments.

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* Studies from this Laboratory, 1884–85, p. 71.

Uranyl acetate.

This salt appeared somewhat more inhibitory in its action than the nitrate, due possibly to its greater acidity The two following series show the extent of action :

$UO_2(C_2H_3O_2)_2 + H_2O$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·4049 gram.	36·44 per cent.	100·0
0·001 per cent.	0·2305	20·74	56·9
0·002	0·1698	15·28	41·9
0·003	trace.		
0	0·4032	36·28	100·0
0·0003	0·4331	38·97	107·3
0·0005	0·3322	29·89	82·4
0·0008	0·3192	28·89	79·6
0·0010	0·2882	25·93	71·4

The presence of 0·003 per cent. of the salt almost entirely stops the action of the ferment, while 0·0003 per cent. decidedly increases amylolytic action. This latter influence is similar to that exerted by many other metallic salts in very small fractions of one per cent. and is doubtless to be attributed, in part at least, to the stimulating action of the acid-proteids formed, or in part, as suggested by Duggan,* to a more complete neutralization of the digestive fluid.

Ammonio uranous sulphate.

With this salt the following results were obtained :

$U(SO_4)_2 + (NH_4)_2SO_4 + H_2O$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·3384 gram.	30·45 per cent.	100·0
0·0003	0·3935	35·41	116·3
0·0005	0·3798	34·18	112·2
0·0008	0·3563	30·40	99·8
0·001	0·3550	30·28	99·4
0·002	0·2951	26·55	87·2
0·003	0·0805	7·24	23·7

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* See Amer. Chem Jour. vol. viii, p. 211.

Here there is to be seen both stimulating and inhibitory action, both quite pronounced; and further, the salt is perfectly neutral, so that such action as is exerted must be due to the salt itself.

Sodio uranic sulphate.

This salt, which like the preceding was exactly neutral to test papers, shows both stimulating and retarding action, but in extent somewhat smaller than that of the uranous salt. Following are the results obtained:

$UO_2SO_4+Na_2SO_4$.	Total amount reducing bodies.	Starch converted	Relative amylolytic action.
0	0·4049 gram.	36·44 per cent.	100·0
0·0003 per cent.	0·4100	36·90	101·3
0·0005	0·4000	36·00	98·8
0·0008	0·4100	36·90	101·2
0·001	0·4117	37·05	101·7
0·002	0·2530	22·77	62·5
0·003	0·2000	18·00	49·4
0·005	trace.		

Potassio uranic oxychloride.

UO_2Cl_2+2KCl $+2H_2O$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·4032 gram.	36·26 per cent.	100·0
0·0005 per cent.	0·3951	35·55	98·0
0·0008	0·4016	36·14	99·6
0·001	0·4083	36·74	101·3
0·002	0·1881	16·92	46·6
0·003	0·1073	9·65	26·6
0·005	trace		

This salt had an acid reaction and its retarding effects are seen to be somewhat more pronounced than that of the two preceding neutral salts.

Ammonio uranic citrate.

$(UO_2)_3(C_6H_5O_7)_2+$ $(NH_4)_3C_6H_5O_7$.	Total amount reducing bodies	Starch converted.	Relative amylolytic action
0	0·4135 gram.	37·21 per cent.	100·0
0·0003 per cent.	0·4298	38·68	103·9
0·0005	0·4016	36·14	97·1
0·0008	0·4049	36·44	97·9
0·001	0·3873	34·85	93·6
0·002	0·4016	36·14	97·1
0·003	0·3843	33·36	89·6

$(UO_2)_3(C_6H_5O_7)_2 + (NH_4)_3C_6H_5O_7$.	Total amount reducing bodies.	Starch converted.	Relative amylolytic action.
0	0·4117 gram.	37·05 per cent.	100·0
0·004 per cent.	0·2378	21·40	57·7
0·005	0·2144	19·29	52·0
0·006	0·2316	21·39	57·7
0·007	0·1845	16·60	44·8
0·008	0·1765	15·88	42·8
0·010	trace		

The action of this salt is mainly a restraining one, but the action is pronounced only with the larger percentages.

As to the way in which these uranium salts diminish the amylolytic action of the ferment, we cannot say definitely. What has previously* been written regarding the action of other metallic salts, under like conditions, is doubtless true here. Loss of amylolytic power is due in part, no doubt, to partial direct destruction of the ferment, as well as to change in the reaction of the fluid. Coupled with this destructive action, however, there must be in addition something in the mere presence of these salts, dependent on chemical constitution, that controls the action of the ferment.

The following table shows the relative acceleration and retardation of the various salts, compared with their respective controls expressed as 100.

2. *Influence on the proteolytic action of pepsin-hydrochloric acid.*

The influence of uranium salts on the proteolytic action of pepsin-hydrochloric acid, was determined by ascertaining the amount of fibrin digested or dissolved in a given time, by a definite volume of a standard, artificial gastric juice, in the presence of varying amounts of the uranium salts. The gastric juice was made by dissolving 10 c.c. of a glycerin extract of pepsin in one litre of 0·2 per cent. hydrochloric acid. The volume of each digestive mixture was 50 c.c.; composed of 25 c.c. of the above mentioned artificial gastric juice and 25 c.c. of 0·2 per cent. hydrochloric acid, containing the necessary amount of uranium salt. The proteid material consisted of purified fibrin, coarsely powdered and dried at 100° C. One gram of fibrin was used in each experiment. The digestive mixtures were warmed at 40° C. for one hour and then the undissolved residue was collected on weighed filters and finally dried at 100–110° C. until of constant weight. The amount of fibrin dissolved is taken as a measure of the proteolytic action.

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* See Studies from this Laboratory, vol. i, 1884–5, pp. 70–75.

TABLE SHOWING RELATIVE ACCELERATION AND RETARDATION OF AMYLOLYTIC ACTION.

Percentage of Salts	0·0001	0·0003	0·0005	0·0008	0·001	0·002	0·003	0·004	0·005	0·006	0·007	0·008
Uranyl nitrate	98·7	93·6	89·4	-----	87·3	-----	75·5	62·5	38·2	-----	----	0
Uranyl acetate	-----	107·3	82·4	79·6	71·4	41·9	0	---	-----	----	---	-----
Ammonio uranous sulphate	-----	116·3	112·2	99·8	99·4	87·2	23·7	-----	-----	-----	----	----
Sodio uranic sulphate	----	101·3	98·8	101·2	101·7	62·5	49·4	----	0	-----	-----	-----
Potassio uranic oxychloride	-----	-----	98·0	99·6	101·3	46·6	26·6	-----	0	----	---	----
Ammonio uranic citrate	-----	103·9	97·1	97·9	93·6	97·1	89·6	57·7	52·0	57·7	44·8	42·8

Following are the results obtained with the various salts:

Uranyl nitrate.

$UO_2(NO_3)_2 + 6H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1353 gram.	86·47 per cent.	100·0
0·025 per cent.	0·1365	86·35	99·8
0·050	0·1378	86·22	99·7
0·100	0·2397	76·03	87·9
0·500	0·5003	49·97	57·8
1·000	0·6638	33·62	38·8

Uranyl acetate.

$UO_2(C_2H_3O_2)_2 + H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1453 gram.	85·47 per cent.	100·0
0·025 per cent.	0·1581	84·19	98·5
0·050	0·1867	81·33	95·1
0·100	0·2052	79·48	92·9
0·500	0·7507	24·93	29·2
1·000	1·0050	0	0

It is to be noticed here, that the retarding action of the acetate, as with saliva, is far greater than the nitrate, a fact which is doubtless dependent in this case on the nature of the acid united with the uranium. Further, it is to be noticed, that the action of uranyl sulphate falls about midway between the action of the nitrate and acetate. These facts accord with views previously* expressed, and show plainly that the extent of the retarding action of salts in general is dependent in part, on the liberation of the acid of the salt and the *digestive power* of the pepsin-*acid* formed. Experiments have shown that nitric acid of appropriate strength, united with pepsin, is about four-fifths as active as hydrochloric acid, while sulphuric acid is only a little more than one-fourth as active as hydrochloric of the same strength and that acetic acid is practically inactive. Hence, the base being the same, acetates, citrates, and other salts, the acids of which are not capable of working with pepsin will most readily retard gastric digestion. This view being correct, uranyl nitrate, sulphate and acetate should retard gastric digestion in just such relative proportion as our experiments show they actually do.

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* See Studies from this Laboratory, 1884–85, p. 94–95.

Uranyl sulphate.

$UO_2SO_4 + 3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1832 gram.	81·68 per cent.	100·0
0·025 per cent.	0·2545	74·55	91·3
0·050	0·2673	73·27	89·7
0·100	0·3155	68·45	83·8
0·500	0·6084	39·16	47·9
1·000	0·8225	17·75	21·7

Ammonio uranous sulphate.

$USO_4 + (NH_4)_2SO_4 + H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1833 gram.	81·67 per cent.	100·0
0·025 per cent.	0 2859	71·41	87·4
0·050	0·3143	68·57	83·9
0·100	0·3742	6·258	76·6
0·500	0·9113	8·87	10·8
1·000	1·0070	0	0

A comparison of the action of the two last salts, shows plainly that the ammonio uranous compound has a far greater inhibitory action than the simple uranyl sulphate.

Ammonio uranic citrate.

$(UO_2)_3(C_6H_5O_7)_2 + (NH_4)_3C_6H_5O_7$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1747 gram.	82·53 per cent.	100·0
0·025 per cent.	0·1795	82·05	99·4
0·050	0·2102	78·98	95·7
0·100	0·2180	78·20	94·7
0·500	0·9055	9·45	11·1
1·000	0	0	0

Sodio uranic sulphate.

$UO_2SO_4 + Na_2SO_4 + 2H_2O$.	Undigested residue.	Fibrin digested	Relative proteolytic action.
0	0·2624 gram.	73·76 per cent.	100·0
0·025 per cent.	0·2666	73·34	99·4
0·050	0·3688	63·12	85·5
0·100	0·4438	55·62	75·6
0·500	0·8131	19·69	26·7
1·000	0·9891	1·09	1·5

In the two last series, the ammonio uranic citrate is noticeable for not causing a gradual diminution in the proteolytic action of the ferment; but on the contrary, it gives rise to a sudden and rapid falling off in proteolytic action, when a certain percentage of the salt is added. The same thing is to be noticed in the case of the uranyl acetate and the reason doubtless lies in the fact that the acid in these two salts is wholly incapable of forming an active compound with pepsin, and thus when a percentage of the salt is added sufficient to use up all of the hydrochloric acid of the gastric juice, digestive action comes to a full stop.

Potassio uranic oxychloride.

UO_2Cl_2+2KCl $+2H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·3063 gram.	69·37 per cent.	100·0
0·025 per cent.	0·2472	75·28	108·0
0·050	0·2123	78·77	113·5
0·100	0·2532	74·68	107·6
0·300	0·2648	73·52	105·9
0·500	0·3578	64·22	92·5

With this salt, unlike any of the preceding, there is to be seen a direct stimulating action on the ferment. Only in the presence of 0·5 per cent. of the salt is there any retarding effect produced. This naturally suggests that possibly uranium *per se*, at least in small fractions of a per cent., has really a stimulating effect on ferment action, but that owing to its combination with acids, in the formation of salts, its apparent effects in the case of pepsin-hydrochloric acid are those due to combination of the normal acid of the gastric juice and liberation of the acid of the uranium salt. In this way only, can we explain the noticeable difference in action of the oxychloride and the other uranium salts. Thus 0·5 per cent. of the former causes but slight diminution in proteolytic action, while with all the other salts, the same percentage causes on an average, a diminution in proteolytic action of at least 50 per cent. This would apply, of course, only to small percentages of uranium, for larger amounts of oxychloride would cause the formation of an indigestible uranium-albumin compound.

Here, however, as in the case of all the salts, the action of any given percentage is constant only under definite conditions. Diminish the amount of ferment, for example, and the amount of dissolved proteid matter consequent thereto, and then the retarding action of the same percentage of uranium salt will be correspondingly increased.

This is well illustrated in the following series of experiments with potassio uranic oxychloride. Using the same percentages of salt as employed in the preceding series, with the same strength of acid, but with onlv half the same content of pepsin extract, and the following results were obtained:

Uranium salt	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·1652 gram.	83·48 per cent.	100·0
0·025 per cent.	0·1982	80·18	96·0
0·050	0·2192	78·08	93·5
0·100	0·2569	74·31	89·2
0·300	0·3486	65·14	78·0

Increasing the percentages of the oxychloride still further, either with this strength of pepsin or the preceding, and there is seen a gradual diminution in the action of the ferment. Compared, however, with the action of the preceding salts, retardation is seen to be quite slow; thus even 2·0 per cent. causes a diminution of proteolytic action amounting to only 50 per cent.

The following table of comparisons shows the relative acceleration and retardation of the various salts compared with their respective controls expressed as 100.

Table showing relative Proteolytic Action.

Percentage of Salts....	0·025	0·05	0·1	0·3	0·5	1·0	2.0
Uranyl nitrate	99·8	99·7	87·9	....	57·8	38·8	....
Uranyl acetate	98·5	95·1	92·9	....	29.2	0	
Uranyl sulphate	91·3	89·7	83·8	....	47·9	2·17	
Ammonio uranous sulphate.	87·4	83·9	76·6	....	10·8	0	
Sodio uranic sulphate	99·4	85·5	75·6	...	26·7	1·5	
Ammonio uranic citrate ...	99·4	95·7	94·7	....	11·4	0	
Potassio uranic oxychloride 1	108·0	113·5	107·6	105·9	92·5		
.... 2	96·0	93·5	89 2	78·0	81·0	66·2	49·4

In retarding proteolytic action, the uranium salts act in part by combining with the proteid matter to be digested, forming a uranium-albumin compound, which is indigestible. Further, in a solution at all concentrated, the uranium salt is liable to precipitate mechanically a portion or all of the pepsin along with the albuminous matter. In addition to this, however, retardation is also due, as already expressed, to liberation of the acid of the salt by the hydrochloric acid of the gastric juice and to the subsequent formation of a pepsin-acid only partially, or not at all, capable of digestive action.

3. *Influence on the proteolytic action of trypsin.*

The method employed in determining the extent of proteolytic action in this case was much the same as in the preceding. The trypsin solution was made as neutral as possible and was prepared from dried ox pancreas, previously extracted with alcohol and ether; 20 grams dry pancreas, extracted with 200 c.c., 0·1 per cent. salicylic acid and ultimately diluted to 1 litre. A little thymol was added to prevent decomposition. 50 c.c. of the trypsin solution were used in each experiment, together with 1 gram of prepared fibrin and the necessary amount of uranium salt.

The first experiment was tried with uranyl nitrate, the mixtures being warmed at 40° C. for six hours. Following are the results:

$UO_2(NO_3)_2 + 6H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2927 gram.	70·73 per cent.	100·0
0·010 per cent.	0·3328	66·72	94·3
0·025	0·3460	65·40	92·4
0·050	0·4198	58·02	82·0
0·100	0·5004	49·96	70·6
0·500	——	0	0

With this salt retarding action is seen to be gradual up to a certain point, and then suddenly all ferment action ceases.

Uranyl acetate.

$UO_2(C_2H_3O_2)_2 + H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4234 gram.	57·66 per cent.	100·0
0·010 per cent.	0·4591	54·09	93·8
0·025	0·5460	45·40	78·7
0·050	0·6094	39·06	67·7
0·100	0·8173	18.27	31·6
0·500		0	0

This series of experiments was warmed at 40° C. for about five hours. The inhibitory action of the salt is seen to be more pronounced than that of the nitrate; indeed, there is to be seen here, the same difference in action noticed in the case of the amylolytic ferment.

Uranyl sulphate.

With this salt, under exactly the same conditions of time and temperature as the preceding, the following results were obtained:

$UO_2SO_4+3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3790 gram.	62·10 per cent.	100·0
0·010 per cent.	0·3863	61·37	98·8
0·025	0·5023	49·77	80·1
0·050	0·6066	39·34	63·3
0·100	0·8083	19·17	30·0
0·500	——	0	0

These results are seen to accord almost exactly with the preceding and show that both salts have an action on this ferment much more pronounced than on pepsin-hydrochloric acid.

Ammonio uranous sulphate.

$U(SO_4)+(NH_4)_2SO_4+H_2O$	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4025 gram.	59·75 per cent.	100·0
0·010 per cent.	0·4031	59·69	99·7
0·025	0·4229	57·71	96·4
0·050	0·4824	51·76	86·6
0·100	0·5743	42·57	72·9
0·500	——	0	0

Sodio uranic sulphate.

$UO_2SO_4+Na_2SO_4+2H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3261 gram.	67·39 per cent.	100·0
0·010 per cent.	0·3414	65·86	97·4
0·025	0·3588	64·12	94·8
0·050	0·3961	60·39	89·5
0·100	0·4462	55·38	82·1
0·500	——	0	0

Ammonio uranic citrate.

$(UO_2)_3(C_6H_5O_7)_2+(NH_4)_3C_6H_5O_7$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3955 gram.	60·45 per cent.	100·0
0·010 per cent.	0·4934	50·66	83·8
0·025	0·5282	47·18	78·0
0·050	0·6618	33·82	55·9
0·100	0·7705	22·95	37·8
0·500	0·7168	28·32	46·8

Potassio uranic oxychloride.

$UO_2Cl_2+2KCl+2H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4234 gram.	56·76 per cent.	100·0
0·010 per cent.	0·4498	55·02	95·4
0·025	0·5224	47·76	82·8
0·050	9·5629	43·71	75·9
0·100	0·7087	29·13	50·8
0·500	——	0	0

With the exception of ammonio uranic citrate, the four last salts experimented with, show about the same degree of inhibitory action; the citrate, however, appears less pronounced in its action than the others. As to the manner in which the uranium salts retard the proteolytic action of the pancreatic ferment, it is probable that the main explanation is to be found in the power possessed by the former of combining with proteid matter in general; combining with and rendering indigestible the albuminous material added to the digestive mixture and perhaps precipitating, or even destroying, the ferment itself. Further, the reason why certain salts appear less active than others is perhaps to be found in the fact that "in the precipitation of albuminous matter by uranium salts, the uranium combines directly with the proteid matter, thus liberating the acid of the salt; and as trypsin is inactive in the presence of free mineral acids, and only partially active in the presence of combined acids (combined with proteids), it follows that an organic salt, such as a citrate, would naturally be less active as a retarding agent, than the nitrate or sulphate.

The following table shows the relative retardation of the various salts expressed in terms of relative proteolytic action:

TABLE SHOWING RELATIVE PROTEOLYTIC ACTION.

Percentage of Salts	0·01	0·025	0·05	0·1	0·5
Uranyl nitrate	94·3	92·1	82·0	70·6	0
Uranyl acetate	93·8	78·7	67.7	31·6	0
Uranyl sulphate	98·8	80·1	63·3	30·8	0
Ammonio uranous sulphate	99·7	96·4	86·6	72·9	0
Sodio uranic sulphate	97·4	94·8	89·5	82·1	0
Ammonio uranic citrate	83·8	78·0	55·9	37·8	46·8
Potassio uranic oxychloride	95·4	82·8	75·9	50·8	0

It is thus seen that uranium salts, in the main, like most other metallic salts, exert a decided retarding influence on the action of the digestive ferments.

XVIII.—THE RELATIVE DISTRIBUTION OF ANTIMONY IN THE ORGANS AND TISSUES OF THE BODY, UNDER VARYING CONDITIONS.

By R. H. CHITTENDEN and JOSEPH A. BLAKE, B.A., PH.B.

ORFILA, many years ago, proved that salts of antimony, like the salts of other metallic poisons, are absorbed and can be detected in the animal tissues and secretions, especially in the liver and kidneys; and further that the absorbed antimony is slowly discharged from these quarters through the medium of the urine. These early results were confirmed by other investigators, notably Danger and Flandin and by Panizza and Kramer, the latter of whom detected antimony, not only in the urine, but also in the blood of a man during a course of tartar emetic.* Orfila's work also indicated that while the elimination of absorbed antimony commences very quickly, it is a comparatively slow process; thus in one instance he stated † that he found antimony in the fat, liver and bones of a dog that had taken, three months and a half before its death, 46·5 grains of tartar emetic during a period of ten days, and that similar results were obtained in a second case in which the interval was four months. Presumably, however, elimination is much more rapid than these figures would seem to indicate.

Dr. Richardson, however, found antimony in abundant proportions in the liver, and in smaller proportions in the kidney and heart, twenty-one days after the last dose of antimony had been taken. As to the relative distribution of absorbed antimony, the experiments of Drs. Nevins and Richardson are the only important ones recorded. Dr. Nevins,‡ experimenting on rabbits, with tartar emetic in doses of 0·5, 1 and 2 grains four times daily, found that the weakest rabbit died after taking 12 grains, the strongest after taking 72 grains of the poison. Other rabbits were killed at varying lengths of time after taking the last dose of poison (31, 14, 4, and 3 days), and in every case antimony was found in large quantity in the liver, in smaller quantities in the spleen and stomach. Antimony was likewise found in the kidneys and urine of those animals that survived for some time, also in the lungs and in those that lived 15 days, in the bones likewise. Dr. Nevins further states, that it was difficult to detect the poison in the muscles and in the blood, but it was found in the bones as late as the thirty-first day after discontinuing the poison.

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* See Christison on Poisons, p. 372.

† Traité de Toxicologie.

‡ See Reese, Manual of Toxicology, p. 259–260 and Woodman & Tidy, Forensic Medicine and Toxicology, p. 128–129.

Dr. Richardson* in 1856, examined the tissues of a dog that died 1 hour and 40 minutes after a solution containing a drachm of tartar emetic had been injected into the cellular tissue. The antimony was found in the following parts, in the order given as to quantity; blood, vomit, rectum, lungs, liver, stomach, bladder, kidneys and small intestines. In a second experiment, a wound in a dog's neck was dressed every morning with tartar emetic ointment, the dog dying at the end of the seventh day. In this case no antimony was found in the brain, but it was found in larger quantities in the liver and spleen than in the other organs. It is very evident, therefore, that tartar emetic, and presumably other salts of antimony likewise, will penetrate all the tissues of the body and that at the same time the antimony is constantly being eliminated by the kidneys. Further than this, the few results recorded indicate nothing definite. As Dr. Richardson well says, "the election of antimony by different parts of the body is as yet an open question; that the liver, however, would appear to be the structure in which it is most collected when the administration is slow and in small doses; and that the elimination of the poison is attempted by all the secreting surfaces." No positive statements can therefore be made regarding the relative distribution of antimony, other than in a general way.

Hence, it has been our object in the present investigation to study somewhat in detail, the relative distribution of antimony in the different tissues of the body under varying conditions; both as to the form of the poison and the manner of its introduction. As with arsenic, so with antimony, the relative proportion of poison found in the different tissues after death may become of considerable medico-legal importance, provided we have sufficient confirmatory data from which to draw conclusions. Particularly is it of importance to know the way in which the form of the poison will influence its distribution. Whether as with arsenic,† the administration of a soluble and diffusible form of the poison will lead to a noticeable accumulation in the brain or nerve tissue in general.

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*See Woodman & Tidy, p 120. Also Dr. W. B. Richardson, Abstract in Amer. Jour. Med Sciences, 1857, vol. 33, p. 266, and B. and F. Med. Chirurg Rev. Oct 1856.

† See Amer Chem Jour., vol. v, p. 8, also Studies from the Laboratory of Physiological Chemistry, S.S.S. of Yale College, 1884–85, p. 141.

1. *The quantitative estimation of antimony.*

There are many methods by which antimony may be detected, even when present in quite small quantities; but there are only a few which yield accurate quantitative results, particularly in the presence of organic matter. In attempting to find a method sufficiently accurate for our purpose, we first tried the method recommended by Orfila, of introducing the final antimony solution into the Marsh apparatus; using for this purpose the form of apparatus and mode of procedure, found so efficacious in the case of arsenic.* In every trial, however, there was a loss of at least 40 per cent. of the antimony; thus in the first case, with an amount of tartar emetic equivalent to 6 milligrams of metallic antimony, only 3·4 milligrams of the metal were recovered, and under exactly such conditions as with arsenic would lead to the recovery of the entire amount. And since in this trial experiment, the antimony was introduced directly into the Marsh apparatus, as tartar emetic dissolved simply in dilute sulphuric acid and without the presence of any organic matter to act as a hindrance, it follows that the loss must be due to retention of a portion of the antimony by the zinc and platinum. A second trial, with the same amount of antimony salt, gave a decided deposit of metallic antimony in the heated tube, but weighing only 3·5 milligrams. In this case the apparatus ran for three hours, but as before, it is evident that only a portion of the antimony was converted into antimoniuretted hydrogen, the remainder undoubtedly being retained either by the platinum used to alloy the zinc, or by the zinc itself. The presence of a small amount of platinum fused in with the zinc, previous to its granulation, does not appear to offer any obstacles to the complete evolution of arsenic as arseniuretted hydrogen. Numerous results obtained by one of us† testify to the accuracy of this statement. Bernstein had also noticed that alloying zinc with platinum or silver, did not hinder the complete evolution of the arsenic, while the addition of a little platinic chloride solution to the acid fluid from which arsenic was being evolved, led to the precipitation of even 50 per cent. of the arsenic present.‡ This probably is the explanation of the low results obtained by Hedden and Sadler in the estimation of arsenic by the Marsh apparatus, in the presence of platinum.§ But with antimony, either the platinum alloyed with the zinc is sufficient to retain fully 50 per cent. of the metal, or as is very probable, the zinc itself causes a precipitation of a portion of the antimony. That this is a point overlooked by most writers on chemical toxicology, is evident from a perusal of the literature on the subject.

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* See Amer Chem. Jour., vol ii., p. 235. † See Amer. Chem Jour , vol. ii, p. 235.

‡ See Dragendorff, Gerichtlich chemische Mittheilung von Giften, p. 334, foot note.

§ See Amer. Chem. Jour., vol. vii, p. 342.

Whether it would be possible to obtain all of the antimony as antimoniuretted hydrogen, in the absence of any metal other than perfectly pure zinc, we cannot say. Certainly as a method for quantitative purposes, it would be too tedious a process to admit of general use, especially where such small amounts of antimony, as in our own experiments, would limit galvanic action to a minimum. That the method is capable of showing the presence of very small amounts of antimony, is unquestionable. Wormley's experiments* are very decided on this point, but evidently only a portion of the antimony will be recovered.

Precipitation of the antimony by hydrogen sulphide and final oxidation by fuming nitric acid and weighing as Sb_2O_4, gave fairly satisfactory results, when the weight of antimony was not less than 10 milligrams of Sb_2O_4. With smaller amounts, the results were far too high, owing probably to the far greater proportional increase of sulphur. Attempts to weigh as sulphide, after fusion of the first hydrogen sulphide precipitate with potassium nitrate and carbonate, likewise gave too high results when the amount of antimony was small. Further, the two latter methods are somewhat unsatisfactory, in that when the amount of antimony is very small, the nature of the final products is such, it is difficult to be certain of the purity of the matter weighed.

Owing to this reason partly, we next turned our attention to the electrolytic method for the separation and determination of antimony, as in this case the appearance of the metallic mirror is in itself a fair guarantee of the nature of the deposit, and its purity is easily proved·

Alex Classen† has shown the accuracy of the method in general quantitative work, where moderate amounts of antimony are present (0·15—0·2 grams Sb). In his experiments, the antimony, in the form of sulphide, was dissolved in ammonium sulphide and the solution then exposed to electrolytic action. The ammonium sulphide solution must be free both from polysulphides and from free ammonia.

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* Micro-Chemistry of Poisons, p. 229.

† Quantitative Analyse durch Elektrolyse. Berichte d. deutsch. chem. Gesell., xvii, p. 2474. See also Alex. Classen and Rob. Ludwig, ibid., xviii, p. 1104.

Classen also found that antimony was deposited quantitatively, when the sulphide was dissolved either in potassium or sodium monosulphide, or in potassium or sodium hydrosulphide. Polysulphides must also be absent in this case, and the potassium and sodium must be quite free from both iron and aluminum, as by long continued electrolytic action, sulphide of iron and aluminum hydroxide may be deposited upon the antimony. Classen also recommends the use of ammonium sulphydrate and a weak current; a strong current tending to cause the separation of the antimony in a pulverulent form, not closely adherent to the platinum.

As it would be necessary in our work, after oxidation of the organic matter, to separate the antimony as sulphide, the above method seemed particularly advantageous, and experiments were therefore tried to ascertain its value when applied to very small quantities. We employed a battery of four moderate sized gravity cells, giving a weak current, and as a rule, exposed the solution to the action of the current for at least 15 hours, as we found better results were obtained by the long continued action of a weak current, than by the quicker action of a more rapid one; particularly in such solutions as we usually had to work with, containing considerable excess of sulphur and some organic matter. The negative pole of the battery was either a small platinum crucible or a platinum dish, while the positive pole was a large piece of platinum foil welded onto a good sized platinum wire. The deposition of the antimony was much more complete, more tightly adherent to the platinum, and as a rule less mixed with sulphur under this arrangement, than when the dish was made to serve as the positive pole; due, doubtless, simply to the broader surface for the deposition of the metal.

Using ammonium sulphide as a solvent for the antimony sulphide, did not give us very good results by electrolysis, the loss being considerable. Much better results were obtained by using a solution of sodium monosulphide, made by saturating one-half of a 15 per cent. solution of sodium hydroxide with hydrogen sulphide, and then adding the remaining half of the sodium hydroxide solution.

The method was tested by precipitating definite volumes of a standard antimony (tartar emetic) solution with hydrogen sulphide, dissolving the metallic sulphide in the sodium monosulphide and then exposing the solutions to electrolytic action. When the separation of the metal was complete, it was found best to wash the deposit with considerable water, without breaking the current; as sometimes, as in the presence of tartaric acid, the separated metal rapidly dissolved on discontinuing the current. The antimony was then finally washed with alcohol, any adherent sulphur lightly brushed off and the dish dried and weighed.

Following are some of the preliminary results obtained:

Standard Sb Solution.	Theoretical Am't Sb	Am't Sb found.	Duration of Electrolysis.
50 c. c.	0·0375 gram.	0·0375 gram.	16 hours.
10	0·0075	0·0077	18
10	0·0075	0·0075	24
10	0·0075	0·0078	18
10	0·0075	0·0074	8
2	0·0015	0·0012	10
1	0·00075	0·0008	3

These results showed the method to be perfectly satisfactory for our purposes, and we, therefore, next tried the separation of small amounts of antimony from organic matter, and its final recovery by the above method.

As there was no doubt that large amounts of antimony could be satisfactorily recovered from organic matter, our experiments were confined mainly to very small quantities. In each experiment, 100 grams of either liver or beef were finely divided and to the so-prepared tissue, a few cubic centimetres of the standard antimony solution were added and the mixture *thoroughly* oxidized with hydrochloric acid and potassium chlorate. After removal of all free chlorine from the filtered fluid, by careful heating, the antimony was precipitated by hydrogen sulphide. This precipitate, which naturally contained, in addition to the sulphide of antimony, considerable sulphur and some organic matter, was then treated as follows: While still moist, after being freed from all hydrochloric acid by thorough washing, it was dissolved in the cold sodium monosulphide solution and then directly subjected to electrolysis. At first, we thought it necessary to free the precipitate from its excess of sulphur and organic matter by solution in ammonium sulphide, evaporation, fusion with potassium carbonate and nitrate, etc., obtaining it finally in the form of sulphide again, free from its former impurities. This, however, we found to be unnecessary; in fact the loss was far greater than the gain. Provided the oxidation with potassium chlorate be a thorough one and the free chlorine entirely removed from the solution, the first hydrogen sulphide precipitate is well adapted for electrolysis directly. It was found best, however, to keep the solution on the battery until all of the sulphur and organic matter was oxidized; that is until the reaction of the fluid had become acid. This took, many times, 30 to 40 hours with our slow current. A more rapid current, would, to be sure, bring about a change in reaction much more quickly, but occasionally under such conditions the antimony would be less closely adherent and loss occur. Then again as the sulphur present was gradually changed into sulphuric acid, the final, strongly acid-reacting fluid, became a good conductor and so at the last, even with our four cells, electrolytic action was quite strong. In washing the deposited antimony, the acid fluid was syphoned out and water continuously added, without breaking the current, until the original fluid was entirely removed.

Following are a few of the results obtained, the antimony being added to 100 grams of tissue in each case.

Standard Sb Solution.	Theoretical Am't Sb.	Am't Sb found.	Duration of electrolysis.
10 c. c.	0·0075 gram.	0·0075 gram.	21 hours.
10	0·0075	0·0072	48
10	0·0075	0·0069	36
10	0·0075	0·0074	24
10	0·0075	0·0064	20
5	0·0037	0·0028	18

The results are certainly not all as close as those obtained in the absence of organic matter, but are perhaps as satisfactory as could be expected under the conditions of the experiment, viz: a large proportional amount of tissue (100 grams), a very small amount of metal and a large volume (say 500 c c.) of fluid to precipitate from, with hydrogen sulphide. Even under the most unfavorable conditions, at least 75–80 per cent. of the antimony introduced into the organic matter was recovered.

We next turned our attention to the obtaining of some convenient and quick method for the direct determination of antimony in urine, or other like organic fluid. Preliminary experiments showed us that antimony, in the form of tartar emetic, could be separated completely from a tartaric acid solution by electrolysis. The separation takes place rapidly, but care must be taken to remove all of the tartaric acid solution by displacement with water, before breaking the current, otherwise the deposited antimony will instantly dissolve. Trial tests repeatedly gave results in close accord with theory. Excess of sodium tartrate, however, appeared to interfere somewhat with separation of the antimony; while the presence of sodium chloride in the presence of an excess of tartaric acid, prevented entirely the separation of the antimony. From a sulphuric acid solution, however, antimony was also deposited quantitatively, and on applying this method to antimoniacal urine, we found it possible to recover the antimony without loss. The following results testify to the accuracy of the method:

In each experiment 25 c.c. of normal urine were employed, to which was added a number of cubic centimetres of the standard antimony solution and then 1 c.c. of pure dilute sulphuric acid, after which the solution was connected with the battery.

Standard Sb Solution in 25 c c urine	Theoretical amount Sb	Amount Sb found	Duration of electrolysis
10 c. c.	0·0075 gram.	0·0075 gram.	48 hours.
10 "	0·0075 "	0·0074 "	24 "
10 "	0·0075 "	0·0074 "	20 "
5 "	0·00375 "	0·0038 "	18 "
5 "	0·00375 "	0·0037 "	18 "
5 "	0·00375 "	0·0036 "	15 "
3 "	0·00225 "	0·0024 "	10 "
2 "	0·0015 "	0·0018 "	20 "
2 "	0·0015 "	0·0016 "	18 "
1 "	0·00075 "	0·0008 "	16 "

Doubtless all of these results could have been obtained equally as well in a very much shorter time, but most of the solutions were connected with the battery at night and allowed to run until morning, or whenever convenient. The method is evidently very accurate when applied in this manner, and we have made use of it very satisfactorily, even with 150 c. c. of urine, using in this case a platinum dish of 200 c. c. capacity as the negative electrode.

2. *Relative distribution of absorbed antimony.*

As already stated, the object sought in the following experiments was to ascertain the relative distribution of antimony under varying conditions; particularly, variations in the form of the poison, as its solubility or insolubility; in the method of introduction, as per mouth, rectum or sub-cutaneously; and lastly in the length of time during which the poison is being taken, whether in one large dose or in many small ones frequently repeated. All of these points we have endeavored to cover in the experiments about to be described.

EXPERIMENT I.

Hypodermic injection of a solution of tartar emetic.

0·120 gram of tartar emetic, dissolved in a little water, was introduced under the skin (right thigh) of a cat weighing 1262 grams.

3:10 p. m., solution injected.
3:16 " vomited copiously.
3:20 " " again, simply mucus.
3:23 " " mucus and bile.
3:38 " " and purged.
3:45 " partially paralyzed.
3:54 " vomited again.
4:30 " much prostrated.
5:10 " dead.

The various organs were then separated and the absorbed antimony determined, according to the method already indicated. Following are the results:

	Total weight, grams	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	52·0	6·35	12·21
Brain	27·5	0·60	2·18
Heart and lungs	32·0	0·70	2·18
Kidneys	12·0	0·15	1·25
Stomach and intestines	74·0	0·80	1·08
Muscle from back	138·0	1·25	0·90
	335·5	9·85	

These figures show the greatest absorption by the liver; the brain stands next, while the muscle tissue appears to have absorbed but a relatively small amount of the antimony. In this connection it must be remembered that two hours only, intervened between the introduction of the poison and the death of the animal, hence it is evident that the brain tissue must have a decided tendency to hold absorbed antimony. The antimony, however, was introduced in the form of a readily soluble salt and under conditions directly favoring rapid and wide-spread distribution. That there is certainly some decided selective action, is evident from the fact that the muscle tissue, which must truly have had as good an opportunity as the brain tissue, retained per 100 grams of substance far less of the poison. Elimination had evidently commenced, for the kidneys contained a decided amount of antimony.

EXPERIMENT II.

Hypodermic injection of a solution of tartar emetic.

In this experiment, a smaller amount of tartar emetic was used than in the preceding; and further, the poison was introduced in three distinct doses, thus allowing longer time for absorption. In fact, the animal lived 22 hours after the first dose, hence the experiment stands in striking contrast to No. 1, in which the animal lived but two hours; while the results, contrasted with the preceding, show plainly the influence of time on the distribution of the poison. Following are the results of the experiment on a rabbit weighing 1295 grams.

Mar. 31, at 5:20 p.m.,	injected under the skin of leg,	0·012 grm.	tartar emetic.
April 1, " 8:45 a.m.,	" "	0·035	"
April 1, " 12:45 p.m.,	" "	0·035	"
	Total,	0·082	

Animal died at 3:05 p. m.

Following is the distribution of the antimony:

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Kidneys	11·5	0·60	5·21
Liver	63·0	1·50	2·38
Brain	9·0	0·20	2·22
Stomach and intestines	98·0	2·00	2·04
Heart and lungs	17·0	0·25	1·47
Muscle from back	106·0	0·70	0·66
	304·5	5·25	

As might naturally be expected, the results indicate a more even distribution of the poison than in the preceding experiment. Although two-thirds as much antimony was used as in experiment No. 1, the liver contains a far smaller proportional amount of the poison than in the preceding experiment, while the kidneys stand first in their content of antimony. Between the brain and the liver, there is but little difference and the experiment plainly substantiates the preceding in showing the tendency of brain tissue, under these conditions, to absorb and retain antimony. In the muscle tissue the percentage of absorbed antimony is almost exactly the same as in No. 1, that is, proportional to the amount of antimony introduced. The animal had evidently lived long enough to admit of a fairly complete distribution of the poison, and elimination having been going on for some time, those parts which had originally contained the most, particularly the liver, had been drawn on to the greatest extent; so that at the time death intervened, the excretory organs, notably the kidneys, were the richest in poison. This fact further indicates that the elimination of absorbed antimony proceeds somewhat rapidly.

EXPERIMENT III.

Hypodermic injection of a solution of tartar emetic.

In this experiment, a cat weighing 1613 grams, had injected under the skin of its hind leg 0·150 gram of tartar emetic in one dose. There was some purging and vomiting, and the animal died in 1¼ hours after the administration of the poison. The object sought in this experiment, which is virtually a repetition of No. 1, with a somewhat larger dose of antimony, was simply to see whether there would be found the same relative absorption of antimony by the liver and kidney as in No. 1, and if by chance there should occur a longer interval of time between the introduction of the poison and death, what then would be the relative amounts of antimony in the two organs. As stated above, the animal lived 4½ hours after the administration of the poison, or 2½ longer than the cat in No. 1. Following are the results of the analysis of the two organs:

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	62·0	2·50	4·03
Kidneys	14·5	0·25	1·72

These confirm to a certain extent the results of No. 1, while at the same time the smaller difference between the amount of antimony contained in the liver and kidneys, as compared with the difference found at the end of two hours (see experiment I), would seem to indicate that the liver had already absorbed its maximum amount, and that at the time of death, elimination was well under way; or in other words, that the removal of the absorbed antimony from the liver had already commenced.

EXPERIMENT IV.

(*a.*) *Hypodermic injection of a solution of tartar emetic.*
(*b.*) *Injection of a solution of tartar emetic per rectum.*

These two experiments were undertaken to ascertain whether the avenue by which the poison was introduced, would influence materially the relative absorption of the antimony. The results, however, although interesting, do not definitely answer the question. Absorption by injection per rectum is so much slower than by hypodermic injection, or the effects produced are so much slower in manifesting themselves, that it is impossible to have the conditions exactly alike in the two cases. Either the time required to produce a given effect, in the case of injection per rectum, will be longer than by hypodermic injection, or else the amount of poison must be correspondingly increased; either of which introduces an objectionable element into the experiment.

(*a.*) Rabbit weighing 1485 grams had injected under its skin 0·80 gram of tartar emetic dissolved in a little water. Injection made at 11:35 a. m. At 3:45 p. m., 4 hours and 10 minutes after the first injection, 0·08 gram more was injected in the same manner. At 4:05 p. m. the animal died.

(*b.*) Rabbit weighing 1512 grams had injected per rectum 0·08 gram of tartar emetic dissolved in a little water. Injection made at 11:45 a. m. At 3:50 p. m. the animal apparently not being affected at all, whereas rabbit (*a*) was strongly under the influence of the poison, 0·160 gram more of the salt was injected per rectum as before. At 5:30 p. m. the animal was still alive, but evidently feeling the effect of the poison. The animal died during the night.

Following are the results of the analysis of the parts from the two rabbits:

RABBIT (*a*) *hypodermic injection.*

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Kidneys	10·2	0·65	6·34
Brain	5·5	0·20	3·63
Liver	54·0	1·30	2·40
Heart and lungs	15·5	0·30	1·93
Stomach and intestines	174·0	1·60	0·92
Muscle	110·0	0·50	0·45
	369·2	4·55	

RABBIT (*b*) *injection per rectum.*

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Stomach and small intestines	172	8·89	15·30
Brain	9	0·40	4·40
Rectum and adjoining intestine	18	0·55	3·05
Liver	54	1·60	2·96
Kidneys	13	0·25	1·92
Muscle	100	1·10	1·11
Urine	20	0·20	1·10
Heart and lungs	17	*trace*	
	403	12·99	

Comparing first, the results obtained from rabbit (*a*) with those of the three preceding experiments, we see at once that the distribution of the antimony is much the same as in No. 2, in which, however, the animal lived nearly 22 hours after the introduction of the first dose of poison and somewhat over two hours after the last. The fatal dose, moreover, in this case was nearly the same in amount as the first dose in experiment IV *a*.

The conditions, however, of this experiment (IV *a*) do not exactly accord with any of the preceding, hence close comparisons cannot well be made. The brain, as in all of the experiments with tartar emetic, contains a proportionally large amount of antimony, while the muscle contains a very small amount. The only thing in this experiment not exactly in accord with the preceding experiments, is the proportionally large amount of poison in the kidneys, as compared with the liver. The only apparent explanation seems to be that, the first dose being small, the liver had quickly reached its maximum absorption and elimination had been rapidly going on; so that at the end of the four hours intervening between the first and second doses of the poison, the kidneys had drawn extensively from the liver, rapidly diminishing its content of the poison. Further, after the second dose of poison, the time before death was so short that the additional absorption by the liver was not sufficient to make up the deficiency, and hence the results found. In this connection, it must be remembered that tartar emetic is very readily soluble and diffusible, and that being injected in solution directly under the skin, its absorption must necessarily be very complete and rapid.

In Rabbit (*b*) the conditions are wholly different from those of the preceding experiments. In all, 0·24 gram of tartar emetic, dissolved in water was introduced into the rectum and 8–10 hours, at least, must have intervened between the administration of the first dose of the poison and death. That the stomach and small intestines should contain the largest proportional amount of antimony is perhaps not at all strange, since the antimony solution would naturally pass rapidly by osmosis through the entire alimentary tract. That this, however, is not the full explanation, is evident, when we compare the amount found in the large intestine with the former. If due simply to osmosis, the percentage amount of antimony would be about the same all through the intestines; hence we must look to some selective action for explanation of the increased amount found in the small intestines. In all of the preceding experiments, the amount of antimony found in the stomach and intestines has been considerably greater than in the muscle tissue. Undoubtedly, the greater vascularity of the former has much to do with its greater content of the metal, but even this is not sufficient to account for all of the antimony found; for whenever blood itself has been analyzed, the amount of antimony has not been large. Unquestionably then, we must assume special absorptive action on the part of the epithelial cells of the stomach and small intestines. In this connection it is well to notice the work of Brinton, who proved that when tartar emetic was injected into the vein of an animal, it was very freely and rapidly eliminated by the stomach. This was also corroborated by Dr. Richardson who, in addition, found that a similar elimination followed the inhalation of antimoniuretted hydrogen.[*] In addition, it may be that absorption of antimony from the alimentary tract goes on slowly and that hence only a portion was removed. This idea has considerable to support it, when we consider the distribution of the absorbed antimony. Remembering that in this experiment, a larger amount of antimony was used than in any of the preceding ones, and that there was apparently ample time for absorption, one cannot help but think that the content of antimony in the remaining tissues and organs is very small. This is very evident, and must be due to one of two causes; either there has been a lack of absorption or else elimination has been going on very rapidly. The brain contains a noticeable amount of antimony, larger than found in any preceding case, while the liver and kidneys both contain a comparatively small amount. The amount of antimony in the kidneys and particularly the amount in the urine, plainly indicates that elimination was going on rapidly; but the fact that the percentage content of antimony in the liver is greater than in the kidneys, would perhaps indicate that at the time of death, absorption was not completed. Such being the case, the only inference to be drawn from the two preceding experiments, is that the introduction of tartar emetic into the rectum leads simply to a much slower absorption and distribution of the antimony than by hypodermic injection, but that there is no essential difference in the relative distribution of the poison under these two conditions.

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[*] Quoted by H. C. Wood, Therapeutics, p. 159.

EXPERIMENT V.

(a.) *Tartar emetic in substance, introduced into the stomach.*

(b.) *Antimonious oxide* (Sb_2O_3) *introduced into the stomach.*

In this experiment there were two objects in view; one was to see the effect of tartar emetic in substance, as compared with the action of the same salt introduced into the system by hypodermic injection or per rectum; the second, to compare the absorption of an *insoluble* compound of antimony (Sb_2O_3) with that of the more soluble tartrate.

In this experiment two dogs were used and the poison was fed to them at regular intervals, in small doses, for a period, in each case, of 17 days. The animals were then killed and the various parts analyzed. The two experiments were exactly alike in every respect, except in the amount of poison administered.

(a.) Dog weighing 7·75 kilos was fed 0·762 gram of tartar emetic during a period of 17 days, in two or three doses daily, the individual doses being small enough not to induce vomiting. The first two days, the dose was 0·016 gram per day, the third 0·020 gram, the fourth 0·030 gram and so on, increasing each day until the last daily dose was 0·085 gram of the poison. The dog was then killed by chloroform, just six hours after the last dose of poison was administered.

(b.) Dog weighing 14·2 kilos was fed 2·073 grams of antimonious oxide, during a period of 17 days, in two daily doses of from 0·032 to 0·125 gram per day. The dog was then killed by chloroform, 18 hours after the last dose of antimony was given.

Following are the results of the analysis of the various parts:

DOG (*a*) *with tartar emetic*—(0·762 gram).

	Total weight, grams	Weight of Sb, milligrams	Sb per 100 grams of tissue, milligrams
Liver	304	17·80	5·85
Salivary glands	11	0·25	2·27
Kidneys	58	1·25	2·15
Brain	76	1·15	1·51
Tongue	36	0·40	1·11
Muscle (thigh)	150	1·60	1·06
Spleen	19	0·15	0·80
Heart	77	0·50	0·66
Lung	140	0·50	0·36
Bone (femur and tibia)	43	0·10	0·23
Blood	130	0·20	0·15
Testes	12	*trace*	
Pancreas	23	*trace*	
	1079	24·05	

DOG (*b*) *with antimonious oxide*—(2·073 grams).

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	452	23·70	5·24
Lungs	140	1·80	1·28
Muscle (fore leg)	157	1·20	0·76
Brain	79	0·40	0·50
Muscle (thigh)	200	0·90	0·45
Kidneys	82	0·10	0·12
Heart	117	*trace*	
Blood	440	*trace*	
	1667	28·10	

In considering these results, we notice first that in dog (*a*) the distribution of the poison is much the same as in the preceding experiments with tartar emetic, viz: the liver, kidneys and brain stand first in their content of antimony. That the liver should contain more per 100 grams than the kidneys, although the animal lived full eight hours after the last dose of poison was taken, is here to be expected, since absorption as a whole would naturally be slower than in some of the preceding experiments; and, further, in this case probably all of the antimony would be absorbed through the portal circulation. In the case of dog (*b*), the conditions are different from any heretofore; we have here an insoluble form of antimony contrasted with a readily soluble and diffusible salt. Solution must necessarily be somewhat slow in this case, but the acid juices of the stomach unquestionably do dissolve and render diffusible, at least a portion of, this form of the poison.

We notice first that the total amount of antimony administered, is fully three times as much as the amount of tartar emetic given, and yet the amount of antimony recovered from the different tissues and organs is but 4 milligrams more than in the case of tartar emetic. This suggests that either considerable antimony is excreted by the kidneys (more than in the case of tartar emetic) or else that considerable passes through the alimentary tract unabsorbed. The dog being confined in a cage of suitable construction, the 24 hours' urine was collected on several occasions and the amount of antimony determined. Thus on one day, when 0·097 gram of antimonious oxide had been administered, following after a daily dose of 0·064 gram, the 24 hours' urine contained 13·5 milligrams of antimony (Sb). Later, at a time when the daily dose was 0·130 gram of the oxide, the 24 hours' urine contained 22·5 milligrams of antimony. Hence it is plain that considerable of the antimony given was being absorbed; but bearing in mind that this latter amount was the largest excreted by the kidneys in any one day, and further that the daily dose of antimony was being increased each day rather than diminished, it is also plainly evident from the amount of absorbed antimony found, that a certain portion must pass through the alimentary canal unabsorbed. Further, the small amount of antimony found in the kidneys supplements this view, as does also the noticeably small amount of absorbed antimony found throughout the body, aside from the liver.

One of the main objects in trying this last experiment was to see what influence the form of the poison would have on its absorption by the brain. With arsenic, it has been plainly demonstrated by one of us,* as well as by other workers in this field, that soluble and readily diffusible forms of arsenic are absorbed by the brain in appreciable quantities, while arsenious oxide for example, no matter whether taken in large or small doses, single or oft-repeated, is never found in the brain other than in mere traces. With antimony we had expected to see something of the same kind. The results, however, although tending in that direction, are not quite as decisive as we should have liked. The antimony found in the brain in the antimonious oxide case is, to be sure, considerably smaller in amount than that found in (*a*), although the dose of antimony given in the former was much larger than in the latter case. But it is also to be seen in the antimonious oxide case, that the amount of absorbed antimony in the brain, although very small, is still greater than the amount found in the kidneys or muscle.

We attempted another experiment in the same direction with rabbits, but as the amounts of antimony found in the brain in both animals were hardly more than mere traces, the results do not give us any additional light on the matter. In spite of the fact that the experiment was a failure, so far as its main object was concerned, we venture to describe it, since it well illustrates in other respects, the greater virulence and diffusibility of tartar emetic. Two rabbits of nearly equal weight were selected, and to one potassium antimony tartrate was fed in gradually increasing doses for a period of 17 days, at the end of which time the animal died with all the symptoms of antimoniacal poisoning. To the other rabbit, antimonious oxide was fed for the same period of time, in doses the same as given to the first rabbit; that is, doses equivalent to the antimony (Sb) contained in the tartar emetic. Each rabbit, therefore, received twice a day, the same equivalent of antimony, and at the end of the 17 days the one rabbit had taken 2·34 grams of tartar emetic, the other 1·08 grams of antimonious oxide. While each rabbit had taken the same amount of antimony, the one which had taken it in the form of potassium antimony tartrate was much more severely affected by the poison. In this case there was severe purging and finally death on the 17th day. In the case of the rabbit fed with antimonious oxide, the only apparent effect of the poison was a loss of appetite and great thirst. This animal was killed with chloroform on the death of the first rabbit. Both forms of antimony were administered as powders, by way of the mouth, in small gelatin capsules.

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* See Studies from this Laboratory, vol. I, for the year 1884–85, p. 141.

Following are the results of the analysis of the various parts from the two rabbits:

RABBIT (*a*) *fed with tartar emetic.*

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	50·0	4·8	9·60
Kidneys	6·7	0·5	7·40
Heart and lungs	18·0	0·4	2·22
Muscle from back	55·0	0·5	0·91
Muscle from legs	79·0	0·3	0·38
Brain	7·7	*trace*	
	216·4	6·5	

RABBIT (*b*) *fed with antimonious oxide.*

	Total weight, grams.	Weight of Sb, milligrams.	Sb per 100 grams of tissue, milligrams.
Liver	57·0	1·3	2·28
Muscle from back	77·0	0·7	0·90
Muscle from legs	100·0	0·7	0·70
Kidneys	8·0	*trace*	
Heart and lungs	16·0	*trace*	
Brain	8·5	*trace*	
	266·5	2·7	

Looking at these results and remembering that each animal received the same amount of metallic antimony, it is evident that tartar emetic is much more completely absorbed than the oxide. With tartar emetic, however, the results are not exactly in accord with the previous ones, obtained with this salt; thus the amount of antimony absorbed by the brain is far smaller proportionally than found hitherto. To be sure, the compound was not in the previous experiments introduced into the stomach of a rabbit in the form of powder, and it is possible that the reason for the difference in the amount of antimony found in the brain in this case and that in the brain of the dog similarly treated, lies in the fact of a slower absorption from the stomach of a herbivorous animal.

XIX.—Influence of Antimonious Oxide on Metabolism.

By R. H. Chittenden and Joseph A. Blake.

The physiological action of antimony has been studied mainly with potassium antimony tartrate, the form in which antimony is most commonly used therapeutically. No experiments, however, appear to have been made, even with this salt, to ascertain the influence of antimony on the metabolism of the body. Gäthgens, however, as quoted by Dr. H. C. Wood,[*] found in some incomplete experiments an increase in the elimination of urea after repeated non-toxic doses of antimony. It is further reported[†] that antimonic acid or other preparations of the metal, when taken in half gram doses daily for about two weeks, cause a diminution in the amount of glycogen in the liver and even a total disappearance of it, and that the liver, kidneys and heart undergo fatty degeneration. Grohe and Mosler[‡] have confirmed the latter and state that in the production of the famous fatty livers, a certain amount of the white oxide of antimony is fed to the geese daily. Aside from these facts, there appears little definite regarding the action of antimony on the physiology of nutrition.

What we have, therefore, endeavored to ascertain in the present experiment is the influence of antimony on metabolism; or particularly, on proteid metabolism as manifested in the excretion of nitrogen, sulphur and phosphorus. Previous experiments[§] have shown that potassium antimony tartrate has a noticeable retarding action on pancreatic digestion; we have not, however, deemed it best in the present experiments to use tartar emetic, as the ready solubility and diffusibility of the compound might cause too rapid absorption and thus lead to speedy toxic action. In spite, therefore, of the fact that we have not made any experiments on the influence of antimonious oxide on digestive action, we have preferred to use the latter in the present experiments, because of its probable slower toxic action and also because it has been so extensively used as a means to induce, or to aid in the production of, fatty degeneration.

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[*] Therapeutics, Materia Medica and Toxicology, p. 156.

[†] See Virchow's Archiv., 1865, Band xxxiv, p. 78.

[‡] Compare H. C. Wood. Therapeutics, p. 161.

[§] Studies from this Laboratory, 1884–85, p. 105.

Our experiments were made on a setter dog, weighing 12·6 kilos. The animal was confined in a suitable cage, so that the excretions could be collected daily without loss. The food consisted of fresh beef and crackers, together with a suitable amount of water. The beef was prepared as follows: About 40 lbs. of fresh beef, freed from fat, tendons, etc., was finely divided by passing through a sausage machine and then dried at a low temperature until it had lost about 75 per cent. of water, and was in a condition suitable for preservation. 50 grams of this preserved meat, together with 75 grams of the sampled crackers, soaked in 300 c.c. of water, were fed to the dog twice daily. The meat, as determined by Kjeldahl's method, contained 12·4 per cent. of nitrogen, while the crackers contained 1·9 per cent. Hence the dog was fed daily 15·25 grams of nitrogen.

On May 11th, the dog was put upon this diet and from the 17th on, the 24 hours' urine was collected daily and analyzed. After a period of two weeks, during which daily analysis of the urine had shown a fairly constant composition, antimonious oxide was added to the diet in the quantities indicated in the table of results; the diet of course continuing the same throughout the length of the experiment.

We deemed it better, as well as more accurate, to measure the influence of the antimony by a daily determination of the total nitrogen, sulphur and phosphorus of the urine, rather than to attempt a determination of urea, uric acid, phosphoric acid, etc. Nitrogen, we determined, according to the method of Kjeldahl,* modified slightly as suggested by Dr. E. H. Jenkins, of the Agricultural Experiment Station, viz: 5 c.c. of the acid urine were placed in a long pear-shaped bulb and evaporated to dryness quickly on a water bath. The residue was heated directly over a small flame with 10 c.c. of pure concentrated sulphuric acid and 0·7 gram of oxide of mercury, until oxidation was almost complete. Then, a little finely powdered potassium permanganate was added, to render the oxidation quite complete. The solution was then diluted, an equivalent amount of potassium sulphide added to convert the mercury into sulphide, and lastly a standard solution of sodium hydroxide, after which the ammonia was driven off by boiling and collected in standard acid.

Total phosphorus and sulphur were determined as follows: 50 c.c. of urine were evaporated in a capacious silver dish with 10 grams of potassium hydroxide and 10 grams of potassium nitrate and the residue heated carefully until the organic matter was completely oxidized. The fused mass was then dissolved in water and diluted to 250 c.c. Of this, 100 c.c., representing 20 c.c. of the original urine, were used for the sulphur, while the second 100 c.c. were used for the phosphorous, determination. For sulphur, the 100 c.c. were acidified with hydrochloric acid and evaporated to dryness on a water bath in order to remove all nitrate and nitrite. The residue was then dissolved in water acidified with hydrochloric acid, and the sulphuric acid precipitated with barium chloride in the usual manner. For phosphorus, the 100 c.c. were acidified with nitric acid, evaporated to dryness, the residue dissolved in water, acidified with nitric acid and the phosphoric acid precipitated with molybdenum solution. This precipitate was then dissolved in a dilute solution of ammonia, the phosphoric acid reprecipitated as ammonio-magnesium phosphate, and the phosphorus finally weighed as magnesium pyrophosphate. Chlorine was determined volumetrically in the usual manner, with a standard solution of silver nitrate, after destruction of the organic matter by fusion with potassium nitrate, etc.

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* Neue Methode zur Bestimmung des Stickstoffs in organischen Korpern. Zeitschrift fur analytische chemie, xxii, 366.

The results, expressed in grams per 24 hours, are shown in the accompanying tables. The 24 hours' urine represents the quantity passed from 9 A. M. of one day to 9 A. M. of the next. As, however, the animal was not always regular in its passage of urine, it frequently happened that the quantity on one day would be very small, while on the next it would be correspondingly increased, without any change in specific gravity, and with a daily average corresponding to the normal, as for example on May 25th and 26th.

In order, therefore, to obviate the difficulty which this irregularity tends to introduce into the results, we have added to the table a daily average of each three days results; a study of which shows plainly that antimonious oxide, in the present experiment at least, does not have any noticeable influence on the excretion of any of the elements determined. Numerically, there is a slight increase in the amount of nitrogen excreted during the taking of the antimony, but the increase is noticeable only in the grand average and is altogether too small to be of much significance. Further, it is to be noticed that the average for the two series does not show any corresponding increase in sulphur. If antimony causes an increased excretion of nitrogen, it means an increase in proteid metabolism, which should in turn give rise to an increased excretion of sulphur and phosphorus. It is to be noticed in the daily results, that the excretion of sulphur nd phosphorus runs parallel with the excretion of nitrogen; an in-

	c. c.		grams.	gram	gram
acid.	462	1024·5	10·526	0·6122	0·5789
acid.	510	1024·5	10·949	0·6587	0·6289
acid.	469	1027·5	11·898	0·7355	0·6716
---- -----	*480*	*1025·5*	*11·124*	*0·6688*	*0·6264*
alkaline.	470	1023·5	9·638	0·5716	0·5606
acid.	466	1027·0	11·904	0·7118	0·6953
acid.	468	1029·5	13·226	0·7646	0·7738
----------	*468*	*1026·6*	*11·589*	*0·6826*	*0·6765*
acid.	406	1030 0	11·492	0·6536	0·6654
acid.	392	1027·0	9·512	0·5599	0·6193
acid.	648	1027·5	16·294	1·2396	0·9709
------ ---	*482*	*1028·1*	*12·432*	*0·8177*	*0·7518*

* On the 22d a portion of the urine was lost.

WITHOUT ANTIMONY—*continued.*

Reaction.	Volume.	Sp Gr.	Nitrogen	Phosphorus.	Sulphur
	c c.		grams	gram	gram
acid.	430	1029·5	11·638	0·7379	0·6888
acid.	414	1028·0	10·593	0·6179	0·6243
acid.	455	1028·0	11·404	0·7421	0·6527
----------	*433*	*1038·5*	*11·211*	*0·6993*	*0·6552*
acid.	500	1027·5	13·254	0·8469	0·6860
acid.	482	1027·5	12·724	0·7753	0·6593
acid.	414	1028·5	11·102	0·6503	0·5884

Chlorine.	Amount of Sb_2O_3 taken
gram 0.7239	grains
0·7203	
0·5871	
0·6771	
0·6720	
0·3792	
0·5126	
0·5212	
0·4574	
0·7852	
0·9329	
0·7251	

Chlorine.	Amount of Sb_2O_3 taken.
gram 0·6191	grains.
0·5422	
0·5411	
0·5674	
0 1878	
0·3922	

WITH ANTIMONIOUS OXIDE.

Date.		Reaction.	Volume.	Sp. Gr.	Nitrogen	Phosphorus.	Sulphur	Chlorine	Amount of Sb O_3 taken
			c. c.		grams	gram	gram	gram	grams
June	6.	acid.	490	1030·0	12·968	0·9011	0·6743	0·6521	0·5 0·5
	7.	acid.	350	1030·0	10·060	0·5961	0·5018	0·4601	0·5 0·5
	8.	acid.	560	1026·5	13·226	0·8146	0·7780	0·6361	0·5 0·5
Daily average			*466*	*1028·8*	*12·084*	*0·7706*	*0·6513*	*0·5827*	
June	9.	acid.	432	1030·0	12·587	0·7576	0·6333	0·4597	0·5 1·0
	10.	acid.	474	1027·5	11·838	0·7068	0·6275	0·6898	0·5 0·5
	11.	acid.	424	1028·0	11·890	0·7131	0·5977	0·3450	1·0 0·5
Daily average...........			*443*	*1028·5*	*12·105*	*0·7258*	*0·6161*	*0·4981*	

WITH ANTIMONIOUS OXIDE—*continued.*

Date.	Reaction.	Volume.	Sp. Gr.	Nitrogen.	Phosphorus.	Sulphur.	Chlorine.	Amount of Sb_2O_3 taken.
		c. c.		grams.	gram.	gram.	gram	grams.
June 12.	acid.	504	1030·0	13·648	0·8550	0·7212	0·3536	0.5 1·0
13.	acid.	416	1027·0	10·645	0·6320	0·5307	0·5972	0·5 0·5
14.	alkaline.	478	1028·5	12·900	0·7490	0·8381	0·3571	1·0 0·5
Daily average...........		*466*	*1028·5*	*12·397*	*0·7453*	*0·6966*	*0·4359*	
June 15.	acid.	404	1030·5	11·471	0·6802	0·6788	0·5563	1·0 1·0
16.	acid.	484	1028·0	12·173	0·7664	0·6414	0·5453	1·0
17.	acid.	432	1027·0	10·935	0·5797	0·6297	0·4326	1·0 1·0
Daily average...........		*440*	*1028·5*	*11·526*	*0·6754*	*0·6499*	*0·5114*	
Average for the series...		*453*	*1028·6*	*12·028*	*0·7292*	*0·6534*	*0·5070*	

crease in the latter is always accompanied by an increase in the two former. In the grand average of the results, however, the slight increase in nitrogen is not accompanied by a corresponding increase in sulphur. In fact, the two series of results, indicate plainly that the antimony was without any material action. The total amount of antimony given, 16 grains of the oxide during 13 days, was certainly sufficient in quantity to have exerted its peculiar influence if possessed of any. The antimony was certainly absorbed, and that too in considerable amount. Thus on the 11th of June the 24 hours' urine contained 13·5 milligrams of metallic antimony; on the 17th, 22·4 milligrams; on the 18th, 17·6 milligrams and on the 20th of June, 15·1 milligrams of metallic antimony. These quantities of absorbed antimony would certainly indicate the presence of sufficient antimony for some decided influence on metabolic action, if any existed. The amount of nitrogen excreted daily, is seen to be considerably below the amount of nitrogen ingested. We did not make daily examinations of the fæcal matter, but such as were made showed plainly that the deficiency in nitrogen was contained mainly in the fæces; thus on the 5th of June the 100 grams of fæces excreted, contained 2·42 grams of nitrogen. At that date, the average amount of nitrogen excreted by the urine was 12·36 grams per day; this amount, added to the fæcal nitrogen makes a total of 14·78 grams excreted, against 15·25 grams ingested; a difference to be found mainly in the hair thrown off, and in part, in the ordinary errors of analysis.

We must conclude, therefore, that small repeated doses of antimonious oxide are without influence on the excretion of nitrogen, sulphur and phosphorus, and that consequently this compound, at least when taken in non-toxic doses, has no action on proteid metabolism.

XX.—ON SOME METALLIC COMPOUNDS OF ALBUMIN AND MYOSIN.

By R. H. CHITTENDEN AND HENRY H. WHITEHOUSE, PH.B.

EVER since Lieberkühn, in 1852, attempted to establish the molecular weight of albumin by preparing and analyzing the copper compound resulting from the action of a soluble copper salt on a solution of egg-albumin, various investigations have been published bearing on the nature and composition of the compounds of albumin with the heavy metals. Before this time even, F. Rose, in 1833, had published an analysis of a copper compound of albumin in which he had found from 1·50 to 1·70 per cent. of cupric oxide, and Mitscherlich, in 1837, published an analysis of a similar albumin compound, in which he found from 2·8 to 3·3 per cent. of cupric oxide, while Lieberkühn's compound contained 4·6 per cent. CuO. Compounds of albumin with other metals have also from time to time been prepared, such as zinc, lead, silver and mercury, and in one or two cases provisional formulæ have been given. The results, however, are to be considered as quite uncertain. With platinum chloride a compound appears to have been formed* of somewhat more certain composition. Aside from the more recent experiments of Ritthausen† on the vegetable albumins (gluten-casein, legumin, etc.), egg-albumin has been the chief albuminous body experimented with, and copper the main metal.

Recent work by one of us (C) on the albumose and globulose bodies, together with work on the products formed from casein and myosin, has led to a partial study of the metallic compounds of these bodies. As a preliminary, however, we found it necessary to study a few of the compounds of egg-albumin, and as the results thus obtained were not in accord with the more recent results of Harnack‡ we have continued our work with egg-albumin and with myosin, the results of which we now present.

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* See Commaile, Moniteur Scientifique, 1866, and Fuchs, in Annalen der Chemie, vol. cli, p. 372.

† Die Eiweisskörper der Getreidearten, etc. Journal für prakt. Chem., vol. xii, p. 361.

‡ Untersuchungen über die Kupferverbindungen des Albumins. Zeitschrift für physiologische Chemie, vol. v, p. 198.

I. EGG-ALBUMIN.

(a) *Copper Compounds.*

In looking over the literature of the subject, it becomes evident at once that the older investigators, owing either to the nature of the compound, to adherent impurities or to faulty methods, were not able to obtain concordant results, since the copper compound of egg-albumin, as prepared and analyzed by six distinct investigators, was found to contain from 1·50 to 5·19 per cent. of CuO. In all of these cases the preparation of the copper compound was essentially the same; a solution of egg-albumin was precipitated with a solution of a copper salt, the precipitate collected, washed thoroughly with water, dried, and the copper determined by simple ignition. Naturally this method, as suggested by Harnack, might be expected to give too high results, since the copper precipitate would unquestionably retain considerable of the inorganic matter of the albumin. Treated in this manner, however, F. Rose,[*] as already stated, found the copper compound to contain from 1·50 to 1·69 per cent. of CuO. Mitscherlich,[†] who held that the copper precipitate was a compound of egg-albumin with the copper salt, found in his preparations 2·8–3·3 per cent. CuO, while Bielitzki,[‡] who demonstrated that the precipitate was an actual compound of albumin with cupric oxide, found in his preparations 4·75–5·20 per cent. of CuO. Lassaigne, as quoted by Harnack, found 4·95 per cent. of CuO, Mulder[§] 4·44 per cent., while Lieberkühn's[‖] preparation contained 4·6 per cent. of CuO. Further, Ritthausen's copper compounds of the vegetable albumins were found to contain from 11·5 to 17·0 per cent. of CuO. These results collectively, would therefore seem to indicate that when egg-albumin is precipitated by a soluble copper salt, the resulting compound does not contain a definite proportion of albumin and cupric oxide, or else that there are a large number of albumin-copper compounds. More recently, however, E. Harnack,[¶] from analysis of fifteen separate preparations, comes to the conclusion that there are two distinct compounds of albumin with copper; one containing 1·35 per cent. of Cu, the other 2·64 per cent. of Cu, indicating as Harnack suggests, a copper albuminate in the first case of the formula $C_{204}H_{320}N_{52}O_{66}S_2Cu$, in which Cu replaces two atoms of hydrogen in the albumin molecule, and in the second case, an albuminate of the formula $C_{204}H_{318}N_{52}O_{66}S_2Cu_2$, in which two atoms of Cu replace four of hydrogen. The results obtained by this investigator would certainly seem to warrant this conclusion, for the analytical data of the different preparations show but slight variations; 1·34–1·37 per cent. in the one case, and 2·18–2·74 per cent. in the other. Further, Harnack worked with nearly ash free preparations, the compounds after their first precipitation and washing being dissolved in sodium carbonate and reprecipitated by careful addition of acid. By repeating this process several times, the ash of the preparation was almost entirely removed, while the relative proportion of copper and albumin was not affected. As to the conditions which determine the formation of one or the other compound, there seems to be little definite other than that in general, the compound with smaller content of copper was obtained when the precipitation took place in the presence of a slight excess of albumin, and the compound with larger content of copper when in the presence of an excess of the copper salt. In no case were the copper salt and albumin solutions mixed in definite proportions, yet in every case one of the two compounds was formed; further, Harnack states that when an amount of copper salt exactly sufficient to form the albuminate is added to a given quantity of albumin, no precipitate results; in other words an excess of the copper salt is necessary to insure a separation of the compound.

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[*] Poggendorff's Annalen, vol. xxviii, 1833.

[†] Muller's Archiv. for 1837, p. 91.

[‡] Dissertation, Dorpat, 1853.

[§] Physiologische Chemie, 1844–51.

[‖] Poggendorff's Annalen, vol. lxxxvi, 1852.

[¶] Loc. cit.

Harnack's results, therefore, differ from those of the preceding investigators in that definite compounds appear to have been formed in every case, and further, in that the compounds contain a lower percentage of copper than found by any other investigators aside from F. Rose. This latter, it will be remembered, found 1·50–1·69 per cent. of CuO; 1·69 per cent. being equal to 1·34 per cent. of Cu, one of the percentages found by Harnack. Harnack further states that the average of the analyses made by other investigators, aside from Rose, show about 4·4 per cent. of CuO, and assuming that the various preparations contained an amount of ash equivalent to about 1 per cent. (which amount Harnack found in his preparations before purification) the percentage amount of cupric oxide would be reduced to about 3·4 =2·7 per cent. Cu, or the amount found by Harnack in his highest copper compound. But as Rose's preparation was made by the simple addition of an aqueous solution of egg-albumin to the copper salt and the copper determined as oxide by simple ignition, it would seem necessary to make the same deduction of 1 per cent. also in this case, which would make Rose's compound contain far less CuO than found by Harnack. Further, Rose* found that the serum of ox blood yielded a similar compound with cupric sulphate, which contained only 1·14 per cent. of CuO or 0·88 per cent. of Cu. Hence there would seem to be little in these earlier investigations to substantiate the results obtained by Harnack. Morner,† however, working with alkali-albuminate, found that on precipitating a solution of alkali-albuminate with cupric sulphate, in the presence of an excess of alkali, he obtained a copper albuminate containing a percentage of cupric oxide corresponding closely with that found by Lieberkühn. When, on the other hand, he precipitated a nearly neutral solution of alkali-albuminate with cupric sulphate, then the percentage of copper in the copper albuminate amounted to only one-third that found by Lieberkühn, or an amount about equivalent to that found by Harnack in his lowest copper compounds. Morner further found that by precipitating a calcium albuminate solution with cupric chloride, the albuminate combined on an average with 2·33 per cent. of CuO, or just one-half the amount required by Lieberkuhn's formula, and considerably less than the amount contained in Harnack's largest copper compounds.

Preparation of the albumin solution.—In our experiments it was thought best, as far as possible, to avoid exposing the albuminate to the action of alkalies, hence especial care was taken to prepare the egg-albumin as free from salts as possible, so that it would not be necessary to purify the albuminate by reprecipitation. The whites of a large number of eggs were finely divided by scissors and by shaking with glass, then mixed with an equal volume of water and thoroughly shaken with air, after which the solution was strained through cloth. Globulin was then precipitated by the addition of dilute acetic acid (the acid added as long as a precipitate formed), the solution finally filtered through paper, after which the filtrate was made exactly neutral with sodium carbonate and again filtered. The fluid so obtained was then dialyzed in running water for many days, a little thymol being added to prevent putrifaction. The fluid finally obtained was perfectly neutral, clear and contained but a small amount of inorganic salts.

In forming the albuminate we employed both cupric acetate and cupric sulphate, using in each case the same volume of albumin solution, but varying the amount of copper salt. The copper salt was generally added as long as a precipitate formed. The albuminate was washed on a pump with water, until no reaction could be obtained with potassium ferrocyanide for copper or with acetic acid and potassium ferrocyanide for albumin. The preparations were dried at 100° C., then powdered and further dried at 110° C., until of constant weight. The percentage of copper was first determined by simple ignition and weighing as cupric oxide. The oxide was then dissolved in dilute nitric acid, the copper precipitated as sulphide with hydrogen sulphide and weighed as subsulphide by ignition in hydrogen gas with a little sulphur. Each series was made from a distinct preparation of albumin and nearly every compound made, was analyzed in duplicate.

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* Loc. cit., p. 139.

† Jahresbericht fur Thierchemie, 1877, p. 8.

Following are the analytical results:

SERIES I.

With $CuSO_4$.

No.	Am't sub. taken.	Wt. CuO.	Per cent. Cu.	Wt Cu_2S.	Per cent. Cu
1*a*	0·5621 gram.	0·0081 gram.	1·13	0·0070 gram.	0·97
b	0·5176	0·0078	1·19	0·0065	0·98
		With $Cu(C_2H_3O_2)_2$.			
2*a*	0·5479	0·0088	1·27	....	-
b	0·5703	0·0091	1·26	0·0078	1·08

SERIES II.

With $CuSO_4$.

No.	Am't sub. taken.	Wt. CuO.	Per cent. Cu.	Wt Cu_2S.	Per cent. Cu
1*a*	0·5273 gram.	0.0067 gram.	1·00	0·0053 gram.	0·79
b	0·7075	0·0083	0·93	0·0068	0·76
		With $Cu(C_2H_3O_2)_2$.			
2*a*	0·5697	0·0081	1·12	0·0071	0·98
b	0·5005	0·0073	1·15	0·0061	0·95

SERIES III.

With $CuSO_4$.

No.	Am't sub. taken.	Wt. CuO.	Per cent. Cu.	Wt Cu_2S.	Per cent. Cu
1*a*	0·6285 gram.	0·0085 gram.	1·07	....	....
b	0·6705	0·0091	1·07	0·0088 gram.	1.04
		With $Cu(C_2H_3O_2)_2$.			
2*a*	0·8302	0·0130	1·24	....	....
b	0·9400	0·0151	1·27	...	....

SERIES IV.

With $CuSO_4$.

No	Amt. sub. taken.	Wt. CuO	Per cent. Cu	Wt. Cu_2S.	Per cent. Cu
1*a*	0·3621 gram.	0·0047 gram.	1·02	0·0038 gram.	0·82
b	0·3478	0·0046	1·03	0·0039	0·89
2*a*	0·4318	0·0060	1·08	0·0040	0·71
b	0·3288	0·0047	1·12	0·0033	0·78
3*a*	0·4083	0·0060	1·15	0·0047	0·90
b	0·4332	0·0064	1·17	0·0053	0·96
4*a*	0·3874	0·0047	1·09	0·0042	0·97
b	0·4289	0·0062	1·14	0·0050	0·90

With $Cu(C_2H_3O_2)_2$.

5*a*	0·6358	0·0090	1·11	0·0073	0·91
b	0·5147	0·0071	1·08	0·0064	0·99
6*a*	0·5321	0·0075	1·12	0·0065	0·95
b	0·5125	0·0073	1·13	-	
7*a*	0·7260	0·0125	1·36	-	
b	0·6916	0·0120	1·37	0·0100	1·15

SERIES V.

With $CuSO_4$.

1*a*	0·4838 gram.	0·0083 gram.	1·36	0·0074 gram.	1·31
b	0·5083	0·0091	1·41	....	

With $Cu(C_2H_3O_2)_2$.

2*a*	0·5044	0·0085	1·32
b	0·5042	0·0084	1·32

From the analyses of these 15 preparations it is to be seen that the percentage amount of metallic copper, determined as oxide by simple ignition, amounts on an average to 1·17 per cent. When, however, the copper is determined as subsulphide, by precipitation with hydrogen sulphide, and thus obtained free from ash, the percentage amount of copper falls on an average to 0·94 per cent. Cu. The preparations thus contain 0·23 per cent. of ash. We were not able to obtain any copper compounds with a much smaller content of ash than this, except by the use of methods which appear to affect the composition of the compound.

Harnack states* that he was able to obtain the copper albuminate quite free from ash by dissolving the freshly precipitated albuminate, after it had been thoroughly washed, in sodium carbonate, filtering and reprecipitating the compound by careful addition of acid. By repeating this process several times the adhering inorganic matter was entirely removed. It seemed questionable, however, whether this treatment might not induce some alteration in the compound.

The two following series of experiments were tried with the intention of throwing some light upon this point.

SERIES VI.

With $CuSO_4$.

No.	Am't sub. taken.	Wt. CuO.	Per cent Cu.	Wt. Cu_2S.	Per cent. Cu.
1*a*	0·6320 gram.	0·0091 gram.	1·15	0·0080 gram.	0·99
b	0·6980	0·0099	1·13	0·0086	0·98
2*a*	0·5428	0·0077	1·12	0·0070	1·01
b	0·5121	0·0077	1·19	0·0061	0·95
3	0·3146	0·0080	2·03	0·0068	1·71
		With $Cu(C_2H_3O_2)_2$.			
4*a*	0·6105	0·0085	1·11	0·0075	0·96
b	0·5836	0·0078	1·06	0·0072	0·99
5*a*	0·5996	0·0087	1·15	0·0081	1·06
b	0·5592	0·0082	1·16	0·0069	0·99
6	0·4139	0·0131	2·51	0·0115	2.19

In this series of experiments all of the preparations, as before, were made from the purified albumin. Nos. 1, 2, 4 and 5 were, after precipitation, simply washed with water until the washings gave no reaction either for copper or albumin. No. 3, after being washed in a similar manner, was dissolved in very dilute sodium carbonate and reprecipitated by neutralization with dilute hydrochloric acid. No. 6 was dissolved up twice in this manner and both preparations were finally washed free from chlorine. A glance at the analyses shows plainly that this treatment has tended to increase the percentage amount of copper in the albuminate; due, doubtless, either to withdrawal of a portion of the albumin by the sodium carbonate, or else to a partial dissociation of the compound by the long continued washing with water.

The first reprecipitation has apparently increased the amount of copper in the compound fully 0·7 per cent., the second reprecipitation 0·5 per cent. more.

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* Loc. cit., p. 202.

SERIES VII.

With $CuSO_4$.

No.	Am't sub. taken.	Wt. CuO	Per cent. Cu	Wt Cu_2S.	Per cent. Cu.
1*a*	0·6326 gram.	0·0091 gram.	1·13		- -
b	0·6156	0·0086	1·10	-	
2*a*	0·4054	0·0081	1·60	0·0063 gram.	1·23
b	0·4995	0·0099	1·58	0·0074	1·19
3*a*	0·3870	0·0068	1·39	0·0049	1·02
b	0·3702	0·0066	1·40	-	- - -

With $Cu(C_2H_3O_2)_2$.

4*a*	0·6175	0·0085	1·08	0·0071	0·95
b	0·6069	0·0082	1·07	0·0077	1·00
5*a*	0·3282	0·0072	1·73	0·0055	1·34
b	0·3755	0·0083	1·75	-	
6*a*	0·7503	0·0132	1·39	0·0104	1·10
b	0·7016	0·0121	1·38	-	- -

This series was prepared in the same manner as the preceding. Nos. 1, 3, 4 and 6 were simply washed with water, while No. 2 was reprecipitated once and No. 5 twice, and both ultimately washed free from all soluble matters. The results show here the same increased percentage of copper, although not so marked as in the preceding series, when the albuminate is dissolved in sodium carbonate and reprecipitated. Further, the percentage of ash is not, as a rule, materially changed by this process; thus in No. 2, where the albuminate was reprecipitated once, the difference in the percentage of copper as determined by simple ignition and by precipitation as sulphide, amounts to 0·37 per cent., while in No. 3*a*, where the compound was not reprecipited at all, the difference is exactly the same.

In precipitating the albuminate, there is formed in the fluid a small amount of either sulphuric or acetic acid. Harnack, to avoid this, states that it is better, after adding the necessary amount of cupric sulphate to the albumin solution, to exactly neutralize the mixture with sodium carbonate. If, however, the greatest care is not exercised and excess of cupric sulphate avoided, even partial neutralization of the fluid will result in the precipitation of a portion of the copper and thus show an apparent increase in the copper of the albuminate. So far, however, as our results show, the small amount of sulphuric acid liberated in the formation of the albuminate does not affect the character of the compound. In the following series, after each precipitation, the mixture was made as near neutral as possible with dilute sodium carbonate and the compounds then filtered and washed thoroughly with water. The copper in this series was determined simply by ignition.

SERIES VIII.

With $CuSO_4$.

No	Am't sub taken.	Wt. CuO.	Per cent Cu
1*a*	0·4273 gram.	0.0068 gram.	1·26
b	0·3867	0·0063	1·29
2*a*	0·3167	0·0049	1·23
b	0·3535	0·0052	1·18
3*a*	0·2418	0·0030	1·00
b	0·3079	0·0039	1·00
4*a*	0·6210	0·0138	1·78
b	0·7091	0·0158	1·78

The results plainly show no appreciable difference in the composition of the albuminate under this change in the conditions, unless in No. 4 where a larger amount of copper is found than usual. It is our opinion, however, that the small amount of acid liberated by the reaction is not sufficient to cause any especial change in the character of the albuminate; neither, probably, does very dilute sodium carbonate in itself change the substance to such an extent that on neutralization it is not precipitated in nearly its original form, or at least that the action in this case is not any greater than that produced by water alone. In fact we are much inclined to the view that the long continued action of water will gradually but surely affect the composition of the albuminate, and that doubtless the change in the composition of the compound noticed in our experiments on solution of the substance in sodium carbonate and reprecipitation is due to the combined action of the alkaline fluid and of water. Harnack states that week-long treatment of the freshly precipitated albuminate with water will gradually cause dissociation of the compound, but that it can be easily and thoroughly washed without any decomposition whatever.

Our experience, however, leads us to question the correctness of this view. Ordinarily, it has taken us an entire day to completely wash the freshly precipitated albuminate, so that the wash-water should give no reaction whatever for copper or albumin. In precipitating the albumin solution with cupric sulphate, the albumin never appears to be completely precipitated and at the same time, as Harnack has observed, it is necessary to add more than the proportional amount of copper salt to obtain any separation of the albuminate. As a result, the filtrate contains considerable albumin and copper, but even after several hours washing on the pump (the filtration is slow at the best) the precipitate still gives up traces of albumin, as shown by acetic acid and potassium ferrocyanide, long after all traces of copper have disappeared. It is not impossible to wash the compound and reach a point where the wash-water contains neither copper nor albumin, but when the washing goes on slowly and the water remains more or less in contact with the albuminate for 24 hours, then frequently the washings will show traces of albumin continuously, without our being able to reach a point where the test fails to give any reaction whatever, or to show any special change in the intensity of the reaction.

The following series of experiments would appear to substantiate this view. The first six were washed for about twelve hours, when no copper reaction could be obtained in the washings and only the slightest reaction for albumin. The last six were washed for sixteen hours, and finally stood over night on wet filters with more or less water on them. At the end of this time, the washings continued to show a reaction for albumin with acetic acid and potassium ferrocyanide, and indeed the reaction appeared to increase rather than diminish in intensity on further washing. The washings contained no copper.

Following are the results of the analyses:

Series IX.—*With* $CuSO_4$.

No.	Amt. sub. taken.	Wt. CuO.	Per cent. Cu.
1	0·4207 gram.	0·0052 gram.	0·99
2	0·4676	0·0058	0·98
3	0·4881	0·0062	1·00
4	0·3162	0·0037	0·91
5	0·2882	0·0032	0·89
6	0·1857	0·0024	1·02
7	0·2892	0·0050	1·34
8	0·4137	0·0060	1.13
9	0·4077	0·0060	1.18

With $Cu(C_2H_3O_2)_2$.

No.	Amt. sub. taken.	Wt. CuO.	Per cent. Cu.
10	0·6067	0·0126	1·64
11	0·6889	0·0111	1·27
12	0·7126	0·0109	1·22

While the difference is not very great, it is a constant difference, and it is to be remembered that the last six compounds differ in no respects whatever from the first six, except in being subjected to the longer action of water.

In comparing now these different results, it is seen that we have not been able to obtain a copper albuminate with a higher content of Cu than 2·19 per cent., and this only as a result of two reprecipitations; a condition, which, from our experience, tends to alter materially the composition of the original precipitate. The average of the results obtained by simple precipitation, show a content of 0·91 per cent. of Cu. A study of the individual results, however, shows too great a variation to believe wholly in the existence of a single, stable copper albuminate. Either there are one or two definite compounds, which, being more or less unstable, are prone to change under varying conditions and thus give rise to the variations in the content of copper noticed, or else there are a number of definite compounds liable to be formed as the conditions are varied, all of which, however, must be more or less unstable. Glancing over the individual results, it is plain that an amount of Cu approximating to 0·96 per cent. is found altogether too frequently to be the result of chance. Doubtless this figure represents most closely the content of copper in the ordinary copper albuminate obtained by simple precipitation, while the majority of the variations from this figure are due mainly to dissociation.

Taking Lieberkühn's formula of albumin, the following copper albuminates would be possible:

$(C_{72}H_{112}N_{18}SO_{22})_3 + Cu - H_2 = 1{\cdot}29$ per cent. Cu.
$(C_{72}H_{112}N_{18}SO_{22})_4 + Cu - H_2 = 0{\cdot}96$ " " "
$(C_{72}H_{112}N_{18}SO_{22})_5 + Cu - H_2 = 0{\cdot}77$ " " "

For the first, in which Lieberkühn's formula for albumin is trebled, the percentage of copper corresponds nearly to the lowest results obtained by Harnack, while in the second formula the percentage of copper accords closely with the average of our results. Whether the weight of the albumin molecule is represented more nearly by the second formula than by the first we have not sufficient data to determine, but certainly our results with the copper albuminate show a lower percentage of copper than would correspond with the first formula. Further, it would appear that the copper albuminates are readily prone to change under slight provocation and that this point, in part, undoubtedly explains the reason for the great variation in the results obtained by so many workers.

(b) *Lead Compounds.*

Lieberkuhn* states that the lead salt of albumin cannot be obtained pure; that the insoluable precipitate formed by the addition of either lead nitrate or basic lead acetate to a solution of albumin, is simply a mixture. With basic lead acetate, Lieberkühn obtained a precipitate containing 17·86 per cent. of lead oxide, while the precipitate formed with lead nitrate contained 12·78 per cent. of lead oxide.

With protein, Mulder† obtained precipitates on the addition of neutral lead acetate and lead nitrate, which contained respectively 12·45 and 12.68 per cent. of lead oxide, while basic lead acetate gave a precipitate containing 30·63 per cent. of lead oxide. Berzelius‡ states that neutral lead acetate precipitates both albumin and blood serum, but that the greater portion of the albumin remains dissolved in the fluid united with acetic acid. Basic lead acetate on the other hand precipitates the albumin completely.

These last statements accord with our own results; with a neutral lead salt only a small precipitate was obtained, the compound being soluble apparently in both excess of the lead salt and of albumin, while with basic lead acetate the albumin seemed completely precipitated.

Further, Berzelius‖ states, on the authority of Mulder, that if a solution of potassium albuminate be made as neutral as possible with acetic acid and then precipitated with lead nitrate, the lead albuminate so obtained contains on thorough drying 5·84 per cent. of lead oxide.

Following are some of the results of our analyses. The compounds were made from thoroughly dialyzed albumin and were washed free from both lead and any excess of albumin. The preparations were dried at 110° C. until of constant weight and the lead was determined first by simple ignition, with addition of a little ammonium nitrate. The lead oxide, after being weighed, was then dissolved in dilute nitric acid, the solution evaporated to a small volume, the lead precipitated with a little sulphuric acid, two volumes of alcohol added, and the lead sulphate finally filtered and washed with 95 per cent. alcohol. The sulphate was then ignited with proper precautions and from the weight obtained, the percentage of lead again calculated.

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* Poggendorff's Annalen, Band lxxxvi, p. 124

† Lehrbuch der Chemie, Berzelius, ix, p. 29. ‡ Lehrbuch der Chemie, ix, p. 43.

‖ Lehrbuch, ix, p. 49.

SERIES I.

With neutral lead acetate.

No.	Amt. Sub. taken.	Wt. PbO.	Per cent. Pb.	Wt. $PbSO_4$.	Per cent. Pb.
1*a*	0·1972 gram.	0·0188 gram.	3·19	0·0209 gram.	2·85
b	0·3992	0·0150	3·48	0·0166	2·83

SERIES II.

With neutral lead acetate.

1*a*	0·5341	0·0183	3·16	0·0216	2·75
b	0·5381	0·0198	3·40	0·0222	2·80

With basic lead acetate.

2*a*	0·5832	0·0460	7·30	0·0580	6·77
b	0·5008	0·0391	7·22	0·0487	6·62

SERIES III.

With neutral lead acetate.

1*a*	0·8057	0·0262	3·01	0·0274	2·32
b	0·6522	0·0211	2·98	0·0226	2·36

With basic lead acetate.

2*a*	0·7290	0·0593	7·55	0·0615	5·74
b	0·7630	0·0631	7·66	0·0609	5·45

SERIES IV.

With neutral lead acetate.

1*a*	0·4121	0·0152	3·42	0·0136	2·25
b	0·4823	0·0176	3·37	0·0178	2·50

With basic lead acetate.

2*a*	0·7234	0·0651	8·34	.....	..
b	0·5365	0·0482	8·33	.....	

SERIES V.

With a large excess of basic lead acetate.

1*a*	0·6822	0·2119	28·81	.....	
b	0·5429	0·1714	29·28	.....	...
2*a*	0·5836	0·1923	30·56	.....	....
b	0·5913	0·1960	30·76	...	....
3*a*	0·5478	0·1896	32·11	......	....
b	0·5427	0·1873	32·02	......	....

These results plainly indicate that more than one compound of lead is formed, especially so with basic lead acetate, the composition being dependent in this case on the amount of lead salt added. With neutral lead acetate, the variations in composition are not so marked and as it is hardly possible to prepare a lead albuminate free from salts, or to eliminate them wholly in the calculations, it is questionable how far the results should be trusted, except in a general way.

The formula $(C_{72}H_{112}N_{18}SO_{22})_4+Pb-H_2$ would require 3·10 per cent. Pb, while $(C_{72}H_{112}N_{18}SO_{22})_5+Pb-H_2$ would require 2·50 per cent. Pb. In the case of the albuminate formed with basic lead acetate, it is to be noticed that the compound made by the addition of a large excess of the lead salt, contains about five times as much lead as the ordinary basic lead compounds.

(c) *Iron Compounds.*

F. Rose* has made iron albuminate, both from egg-albumin and from the serum of ox-blood, by the simple addition of ferric chloride to the albumin solution. Two preparations made from egg-albumin yielded respectively 2·79 and 2·88 per cent. of ferric oxide. Rose found the albuminate, when freshly precipitated, easily soluble both in excess of ferric chloride and in excess of the albumin solution.

Our preparations were made wholly from dialyzed albumin, and when so prepared and thoroughly washed the compound was found almost wholly free from adhering salts, so much so that after a few trials we deemed it unnecessary to make the determinations of iron other than by simple ignition and weighing as ferric oxide.

Following are some of our results:

SERIES I.

With Fe_2Cl_6.

No.	Amt. sub. taken.	Wt. Fe_2O_3.	Per cent. Fe_2O_3.	Per cent. Fe.
1*a*	0·7038 gram.	0 0094 gram.	1·33	0·92
b	0·6430	0·0086	1·33	0·93

SERIES II.

No.	Amt. sub. taken.	Wt. Fe_2O_3.	Per cent. Fe_2O_3.	Per cent. Fe.
1*a*	0·4683	0·0052	1·11	0·76
b	0·3854	0·0042	1·08	0·75

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* Poggendorff's Annalen, xxviii, p. 140, 1833.

SERIES III.

No.	Amt. sub. taken	Wt. Fe_2O_3.	Per cent. Fe_2O_3.	Per cent Fe.
1a	0·1710	0·0071	1·50	1·01
b	0·5329	0·0083	1·55	1·08
2a	0·7387	0·0100	1·36	0·91
b	0·6355	0·0087	1·36	0·91

SERIES IV.

1a	0·1862	0·0063	1·30	0·90
b	0·5558	0·0073	1·31	0·91
2a	0·5115	0·0070	1·36	0·95
b	0·4951	0·0066	1·33	0·92
3a	0·1505	0·0063	1·39	0·97
b	0·1610	0·0066	1·13	0·99
4a	0·4817	0·0071	1·17	1·01
b	0·4571	0·0065	1·41	0·98
5a	0·4819	0·0065	1·34	0·93
b	0·3816	0·0051	1·33	0·93
6a	0·4086	0·0059	1·15	1·00
b	0·3392	0·0048	1·11	0·97
7a	0·3814	0·0050	1·32	0·91
b	0·4107	0·0053	1·29	0·90
8a	0·4452	0·0058	1·30	0·91
b	0·4833	0·0065	1·31	0·92

These results show a fairly close agreement with one single exception, in which case the percentage of iron is nearly 0·25 below the average. The average percentage, moreover, of ferric oxide is just about one-half that found by Rose. Further, the average percentage of iron (Fe) corresponds very closely with the average percentage of Cu in the copper albuminate. Eliminating one compound with only 0·75 per cent. of iron, the average content is seen to be 0·95 per cent.

$$(C_{72}H_{112}N_{18}SO_{22})_4 + Fe \quad H_4 = 0{\cdot}86 \text{ per cent. Fe.}$$
$$(C_{72}H_{112}N_{18}SO_{22})_3 + Fe \quad H_3 = 1{\cdot}14 \text{ per cent. Fe.}$$

As the iron was determined by simple ignition it would be expected that the amount found would exceed the theoretical amount somewhat; hence the first formula, assuming Lieberkühn's formula to be correct, would be more closely in accord with our results. The results obtained indicate further, that the iron albuminate is a much more stable compound than the copper albuminate, less liable to change and less readily affected by water.

(d) *Zinc Compounds.*

With zinc we made but a few experiments and those mainly to see whether the low percentage of iron found in the iron albuminate would be substantiated by a corresponding percentage of zinc in the zinc albuminate. Lieberkühn has prepared and analyzed a zinc albuminate, made by the action of zinc sulphate on a neutral solution of alkali albuminate, and he found the compound to contain 1·66 per cent. of zinc oxide.

Our preparations were made by the action of a similar zinc salt on a solution of purified and dialyzed albumin. Following are the results obtained with two preparations made from two distinct lots of albumin:

No.	Amt. sub. taken.	Wt. ZnO.	Per cent. ZnO.	Per cent. Zn.
1*a*	0·2424 gram.	0·0031 gram.	1·27	0·98
b	0·2838	0·0034	1·21	0·97
2*a*	0·2166	0·0023	1·06	0·83
b	0·2354	0·0025	1·08	0·86

The average of these two results shows a composition proportional to that found in the case of the iron albuminate and suggests plainly that if we have to deal in these cases with a single albuminate of constant composition, the percentage amount of metal is much smaller than formerly was supposed.

Further, the percentage of zinc found accords closely with the theoretical amount for a zinc albuminate formed on the type of the copper compound.

$$(C_{72}H_{112}N_{18}SO_{22})_4+Zn-H_2=0·99 \text{ per cent. Zn.}$$

(e) *Uranium Compounds.*

N. Kowalewsky* has recently called attention to the use of uranic or uranyl acetate as a reagent for albuminous matter, and has shown that it is not only a good precipitant of albumin at ordinary temperatures, but also that it is an extremely delicate one. Further, Kowalewsky states that the uranyl-albumin compound on ignition leaves a dark, olive green ash, composed of the green uranoso-uranic oxide, U_3O_8. A determination of the amount of this ash in several preparations showed 12·09 to 13·4 per cent., presumably of U_3O_8.

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* Essigsaures Uranoxyd, ein Reagens auf Albuminstoffe. Zeitschrift für Analytische Chemie, 1885, p. 551.

Our preparations were made by adding uranyl nitrate to the prepared albumin solution and washing the precipitated albuminate until all excess of uranium was removed. The uranium in the dried preparation was determined as uranoso-uranic oxide (U_3O_8) by simple ignition. The results show a fairly close agreement, but they are undoubtedly somewhat too high, owing to a small amount of adherent ash.

With $UO_2(NO_3)_2$.

No.	Amt. sub. taken.	Wt. U_3O_8.	Per cent. U_3O_8.	Per cent. U.
1*a*	0·5980 gram.	0·0324 gram.	5·41	4·59
b	0·5193	0·0281	5·41	4·59
2*a*	0·8219	0·0428	5·20	4·41
b	0·8081	0·0418	5·17	4·38
3*a*	0·4392	0·0251	5·71	4·81
b	0·5330	0·0303	5·68	4·81
4*a*	0·8183	0·0427	5·22	4·43
b	0·7576	0·0394	5·20	4·41
5	0·6985	0·0367	5·25	4·46
6*a*	0·4269	0·0247	5·78	4·90
b	0·5196	0·0313	5·70	4·83

These results plainly do not accord at all with Kowalewsky's. On the other hand they do agree fairly well with each other, and would seem to indicate a reasonably constant composition of the uranyl-albumin precipitate.

The average of the results obtained, accords most closely with the formula

$$(C_{72}H_{112}N_{18}SO_{22})_3 + U - H_6$$

which requires 4·73 per cent. U.

(f) *Mercury compounds.*

By the addition of an excess of mercuric chloride solution to an aqueous solution of egg-albumin, an albuminate of mercury is formed, insoluble in excess of the mercury salt. The compound can be easily filtered and admits of thorough washing with water. Rose first proved that the precipitate formed as above, is a compound of mercury with albumin, instead of a compound of the mercury salt with albumin as supposed by Bostock and Orfila.

We have made a few preparations of the albuminate by adding a moderately strong aqueous solution of mercuric chloride to portions of the dialyzed albumin solution and washing the precipitates thoroughly with water. The mercury in the albuminate was determined by ignition in a combustion tube with quick lime, with a posterior layer of calcium carbonate and sodium bicarbonate.[*]

The mercury distilled, was collected in water and after thorough washing with alcohol to remove hydrocarbons, etc., was dried and weighed.

Following are the results of our analyses of the several preparations made.

No	Amt Sub. taken.	Wt Hg	Per cent Hg.
1*a*	0·8050 gram.	0·0226 gram.	2·80
b	0·8732	0·0274	3·13
2*a*	0·7637	0·0215	2·82
b	0·7357	0·0201	2·73
3*a*	0·5088	0·0150	2·96
b	0·6261	0·0167	2·66
4*a*	0·9152	0·0300	3·28
b	0·8503	0·0270	3·17
5*a*	0·8492	0·0218	2·56
b	0·8610	0·0237	2·75
6*a*	0·9674	0·0284	2·93

The average content of mercury is 2·89 per cent. The theoretical amount for $(C_{72}H_{112}N_{18}SO_{22})_4+Hg-H_2$ is 3·00 per cent.

(g) *Silver compounds.*

Silver nitrate is a well known precipitant of albumin, and Lieberkühn,[†] many years ago, assigned to silver albuminate a definite formula, calling for 6·67 per cent. of silver oxide. The preparation made by him from egg-albumin was found to contain 6·55 per cent. of silver oxide = to 6·27 per cent. of Ag. Mulder,[‡] likewise, working with alkali-albuminate, found that by neutralizing the solution as nearly as possible with acetic acid, and then precipitating with silver nitrate, the silver albuminate so prepared contained 6·14 per cent. of silver oxide.

Fuchs[§], using ordinary egg-albumin instead of alkali-albuminate, found only half as much silver (3·28 per cent. Ag), while O. Loew,[‖] working with purified egg-albumin, found still smaller percentages of silver in the albuminate made by him. Using an albumin solution purified simply by three days' dialysis, Loew found that a 1 per cent. solution of silver nitrate gave no precipitate whatever in a 5 per cent. solution of albumin. On adding a little dilute sulphuric acid to the albumin solution, however, and then pouring the mixture into the silver solution a precipitate was obtained, which on thorough washing and drying was found to contain 2·17 per cent. of Ag; while a second preparation made by using a little less sulphuric acid contained 2·40 per cent. of Ag. By precipitating the albumin solution directly with a 5 per cent. solution of silver nitrate, without the addition of any acid, the albuminate was found to contain in one case 4·39 per cent. Ag, in a second case 3·91 per cent. Ag. Dissolving the freshly precipitated albuminate formed in this manner, in dilute ammonia and then reprecipitating it by the addition of dilute sulphuric acid to slight acid reaction, the albuminate was found to contain 4·64 per cent. of Ag.

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[*] See Fresenius, Quantitative Chemical Analysis.

[†] Poggendorff's Annalen, 1852, vol. clxii, p. 123.

[‡] See Berzelius' Lehrbuch der Chemie, vol ix, p. 49

[§] Annalen d. chem. u. Pharm., Band cli, p. 372.

[‖] Pflüger's Archiv fur Physiologie, Band xxxi, p. 393; Ueber Eiweiss und Pepton.

Loew sees in these results a confirmation of Harnack's views as to the copper albuminates, and an assurance that the molecular weight of albumin corresponds to Lieberkühn's formula three times enlarged.

Using an albumin solution purified as in our previous experiments and adding to it a 10 per cent. solution of silver nitrate as long as a precipitate was formed, four distinct series of albuminates were made* representing four distinct preparations of egg-albumin. These were all washed free from silver and also from any adhering albumin, dried at 110° C. until of constant weight and the silver determined by simple ignition.

Series I.

No.	Amt. Sub. taken.	Wt. Ag.	Per cent. Ag.
1*a*	0·5900 gram.	0·0242 gram.	4·10
b	0·5625	0·0230	4·08
2*a*	0·5760	0·0230	4·11
b	0·7518	0·0305	4·01
3*a*	0·9005	0·0362	4·02
b	0·7973	0·0325	4·07

Series II.

No.	Amt. Sub. taken.	Wt. Ag.	Per cent. Ag.
1*a*	0·5859	0·0245	4·18
b	0·6967	0·0290	4·16
2*a*	0·9473	0·0385	4·06
b	0·6621	0·0270	4·07
3*a*	0·6455	0·0266	4·12
b	0·7000	0·0285	4·07

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* The silver compounds were all made and analyzed by Mr. T. S. Bronson of this laboratory.

SERIES III.

1*a*	0·5860	0·0239	4·07
b	0·6949	0·0284	4·08
2*a*	0·7090	0·0290	4·09
b	0·9053	0·0371	4·13
3*a*	0·5980	0·0247	4·13
b	0·8000	0·0328	4·10

SERIES IV.

1*a*	0·6217	0·0300	4·88
b	0·6810	0·0331	4·86
2*a*	0·5509	0·0270	4·90
b	0·5626	0·0278	4·86
3*a*	0·6955	0·0396	5·69
b	0·7515	0·0430	5·72

The figures show a far smaller content of silver in all of the preparations than found by Lieberkühn or Mulder. In three of the series, there is seen a constancy of composition which is quite noticeable and, further, a close agreement with the second result obtained by Loew on adding a 5 per cent. solution of silver nitrate to the albumin. In the last series, however, the percentage of silver is somewhat higher, possibly owing to incomplete dialysis of the chlorides and phosphates from the albumin solution. These figures, however, are not much higher than the highest figures obtained by Loew.

A silver salt of albumin, of the composition $(C_{72}H_{112}N_{18}SO_{22})_3 + Ag_2 - H_2$ would contain 4·28 per cent. of Ag, and while our results certainly approximate to this figure, there is variation enough to indicate an equal possibility of a mixture of two or more compounds. With a molecule of the size of the albumin molecule, it is possible by doubling or otherwise, to obtain a formula corresponding to almost any percentage of metal found. And inasmuch as every variation* in the method of preparing the albuminate tends to alter its composition, it seems worse than useless at present, to lay much stress on the exact constitution of the silver albuminate. A large number of albuminates are of course possible, but until we know more definitely how to separate one from another, we have no guarantee of the simple nature of any one.

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* Loew states that he has prepared a silver albuminate containing 10·7 per cent Ag, corresponding nearly to 6 atoms of silver, and that it is possible to prepare albuminates still richer in silver.

Examining now, all of the results obtained, we find the following average composition of the albuminates studied:

Copper compound,	0·91	per	cent.	Cu
Iron compound,	0·95	"	"	Fe*
Zinc compound,	0·91	"	"	Zn
Lead (neutral salt) compound,	2·56	"	"	Pb
Uranyl compound,	4·60	"	"	U
Silver compound,	4·09	"	"	Ag†
Mercury compound,	2·89	"	"	Hg

Accepting Lieberkuhn's formula of albumin as correct, then the following formulæ accord most closely with the above percentages.

$(C_{72}H_{112}N_{18}SO_{22})_4 + Cu - H_2$	requires	0·96	per cent.	Cu
$(C_{72}H_{112}N_{18}SO_{22})_4 + Fe - H_3$	"	0·86	"	Fe
$(C_{72}H_{112}N_{18}SO_{22})_4 + Zn - H_2$	"	0·99	"	Zn
$(C_{72}H_{112}N_{18}SO_{22})_5 + Pb - H_2$	"	2·50	"	Pb
$(C_{72}H_{112}N_{18}SO_{22})_4 + U - H_4$	"	1·73	"	U
$(C_{72}H_{112}N_{18}SO_{22})_4 + Ag - H$	"	4·28	"	Ag
$(C_{72}H_{112}N_{18}SO_{22})_4 + Hg - H_2$	"	3·00	"	Hg

We do not, however, lay much stress upon the accuracy of these formulæ. The results obtained in our study of these metallic compounds do by chance accord with them, and inasmuch as Loew and Harnack are disposed to treble Lieberkühn's formula for albumin, on the basis of the composition of the copper and silver albuminates, made by them respectively, we present our results as evidence that there are equally good grounds for quadrupling the above formula.

We believe, however, that with the majority of these albuminates it is possible to form a large variety of compounds with the same metal, by simply modifying the conditions of precipitation. This is evidenced by Loew's results with silver albuminate and our own with lead and copper, and since a great variety of compounds are possible, it is equally possible that in many cases we may have to do with mixtures of such compounds, which would account for the great variability in composition noticed in some of the albuminates and for the lack of agreement in the results obtained by different workers. Coupled with this, in some cases, is the undoubted tendency of the compounds to dissociation.

II. *Myosin.*

The myosin employed was prepared from ox flesh, by extraction with a 15 per cent. solution of ammonium chloride, after the tissue had been thoroughly freed from salts and soluble albumin by long continued extraction with water. The myosin was separated from the ammonium chloride solution by dialysis, being obtained in this manner as a semi-gelatinous mass, readily soluble in salt solutions. In order to form compounds with the various metals, it was found best to use a solution of myosin in 5 per cent. ammonium chloride, the metallic compound when formed being washed with water until the washings gave no reaction for chlorine with silver nitrate. The compounds were then dried, first at 100° C., then at 110° C., until of constant weight. Control experiments with the metallic salt and ammonium chloride alone, invariably failed to give any precipitate whatever.

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* Excepting one very low result. † Excepting the last series of compounds.

No systematic attempt has apparently been made to study any of the metallic compounds of myosin; in fact, few statements are to be found regarding the existence of such compounds. Danilewsky[*] some time ago, showed that myosin would combine with free mineral acids, uniting with them so that with tropæolin 00 no reaction for free acid could be obtained. With strong bases, however, according to Danilewsky, myosin does not probably combine, and the statement is further made that a small amount of calcium oxide ordinarily exists loosely combined with myosin, which calcium by coagulation of the myosin is liberated. Further, Danilewsky found that on adding platinum chloride in excess, to a dilute hydrochloric acid solution of myosin, a myosin-platinum chloride compound was precipitated, which after washing with water and alcohol and then drying at 100–105° C., contained 9·46 per cent. of platinum and 7·26 per cent. of chlorine. With copper, iron and similar salts we have not been able to obtain any precipitate in a hydrochloric acid solution of myosin. By adding, however, a solution of a metallic salt of such a nature that it does not react with ammonium chloride, to an ammonium chloride solution of myosin, a precipitate is produced, which as our experiments show, is ordinarily a compound of myosin with the metal or metallic oxide. This is readily seen by adding either zinc sulphate or ferric chloride to such a solution of myosin and then washing the precipitates with water, until the washings give no reaction for chlorine or for sulphuric acid. On now warming the iron precipitate with dilute nitric acid, a solution will be obtained, giving a distinct iron reaction but no reaction with silver nitrate for chlorine. Similarly on warming the zinc precipitate with hydrochloric acid, the solution gives no reaction for sulphuric acid with barium chloride.

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[*] Zeitschrift für physiologische Chemie, v, p. 160.

With cupric sulphate and cupric acetate the same is ordinarily true. It is possible, however, to prepare a myosin-copper compound in which cupric sulphate appears to unite directly with the myosin.

(*a.*) *Copper compounds.*

By adding either cupric sulphate or cupric acetate to a neutral ammonium chloride solution of myosin, a heavy greenish colored precipitate is obtained, which when freshly formed and after thorough washing with water, so that the washings are entirely free from chlorine and from copper, shows the following reactions. It is insoluble in moderately strong nitric, hydrochloric or sulphuric acid. The compound, however, is immediately broken up by the action of acids, the copper being completely removed, leaving the myosin as an insoluble residue having in the case of nitric acid a yellow color, and in the case of hydrochloric and sulphuric acids a white color. In acetic acid, the compound is more soluble, first, however, becoming semi-gelatinous. In ammonium hydroxide, the compound dissolves slowly or partially, taking on a blue color. In dilute sodium hydroxide, the compound swells up, takes on a purple color, but does not dissolve. In dilute sodium carbonate, the substance is likewise insoluble, but swells up and turns of a bluish color.

Following are the results of the analyses of the various preparations made. The compounds were in every case composed simply of the metallic oxide and myosin. Copper was determined, as in the case of the albumin compounds, by simple ignition and weighing as oxide. In order to ascertain how much ash was retained by the myosin compound, a few duplicate determinations of copper were made by dissolving the oxide after ignition, and precipitating the copper as sulphide and weighing as subsulphide, after ignition with a little pure sulphur in a current of hydrogen gas.

Series I.

With $CuSO_4$.

No.	Amt Sub. taken	Wt. CuO.	Per cent. Cu.	Wt. Cu_2S.	Per cent. Cu.
1*a*	0·5426 gram.	0·0074 gram.	1·08	0·0056 gram.	0·81
b	0·7176	0·0097	1·07	0·0073	0·80
2*a*	0·6359	0·0087	1·08	0·0071	0·88
b	0·5738	0·0078	1·08	0·0061	0·83

No.	Amt Sub taken	Wt. CuO.	Per cent. Cu.	Wt. Cu_2S.	Per cent. Cu.
3*a*	0·5423 gram.	0·0072 gram.	1·05	0·0051 gram.	0·73
b	0·6241	0·0082	1·05	0·0063	0·80
4*a*	0·5771	0·0109	1·50		
b	0·4455	0·0081	1·43		
5	0·3527	0·0046	1·02		
6*a*	0·4401	0·0062	1·11		
b	0·5708	0·0082	1·13		

SERIES II.

With $CuSO_4$.

No.	Amt. Sub. taken.	Wt. CuO.	Per cent. CuO.	Per cent. Cu
1*a*	0·7425 gram.	0·0059 gram.	0·79	0·63
b	0·7463	0·0060	0·81	0·64
2*a*	0·7785	0·0057	0·73	0·57
b	0·7767	0·0056	0·72	0·58
3*a*	0·5488	0·0052	0·94	0·74
b	0·4836	0·0041	0·91	0·73
4*a*	0·9477	0·0085	0·89	0·74
b	0·8150	0·0074	0·91	0·73

With $Cu(C_2H_3O_2)_2$.

5*a*	0·8522	0·0133	1·56	1·24
b	0·8466	0·0138	1·62	1·29
6*a*	0·6589	0·0124	1·88	1·50
b	0·6572	0·0123	1·87	1·50
7*a*	0·8673	0·0156	1·79	1·42
b	0·7120	0·0129	1·81	1·44

SERIES III.

With $CuSO_4$.

No.	Amt. Sub. taken.	Wt. CuO.	Per cent. CuO.	Per cent. Cu.
1*a*	0·7213 gram.	0·0171 gram.	2·37	1·88
b	0·6584	0·0161	2·44	1·94
2*a*	0·7564	0·0168	2·22	1·77
b	0·6817	0·0156	2·29	1·83
3*a*	0·7820	0·0189	2·41	1·91
b	0·6213	0·0154	2·48	1·97
4*a*	0·7137	0·0144	2·01	1·61
b	0·5863	0·0124	2·11	1·68
5*a*	0·7732	0·0164	2·12	1·68
b	0·7407	0·0161	2·18	1·74
6*a*	0·7447	0·0183	2·45	1·96
b	0·7429	0·0188	2·53	2·01

With $Cu(C_2H_3O\,)_2$.

No.	Amt. Sub. taken.	Wt. CuO.	Per cent. CuO.	Per cent. Cu
7*a*	0·7360 gram.	0·0161 gram.	2·18	1·73
b	0·7082	0·0154	2·17	1·73
8*a*	0·7789	0·0171	2·21	1·78
b	0·8111	0·0181	2·23	1·77
9*a*	0·7376	0·0169	2·29	1·81
b	0·6155	0·0138	2·24	1·78
10*a*	0·7815	0·0191	2·44	1·94
b	0·8774	0·0213	2·43	1·93
11*a*	0·8048	0·0170	2·11	1·67
b	0·8308	0·0174	2·10	1·67
12*a*	0·6780	0·0150	2·21	1·75
b	0·7695	0·0168	2·18	1·74

Comparing these results with one another, there is to be seen a very noticeable lack of agreement in composition, and further it is to be seen that the myosin-copper compound has on an average a somewhat higher content of copper than the albumin-copper precipitate. The average composition of all the copper myosins shows about 1·42 per cent. of Cu, and deducting 0·25 per cent. of ash, the average content of Cu would be 1·17 per cent. Examining the individual results, it is apparent that the compounds made from the same myosin solution are approximately, at least, the same in composition and without doubt the difference in the composition of compounds made from different myosin solutions is due to variation in the concentration of, and possibly also in the reaction of, the myosin-containing fluid. It would appear as if variations in the conditions of precipitation made a greater difference in the case of the myosin-copper compounds than in the compounds of copper with albumin. Several times, also, we have found that our myosin-copper precipitate contained sulphuric acid, even after thorough washing and when the wash-water was proved to be entirely free from any reaction with barium chloride.

One such compound, after drying at 110° C., was analyzed with the following results:

0·7240 gram substance* gave 0·0343 gram $BaSO_4$ = 1·63 per cent. SO_3.
0·8420 gram substance gave 0·0331 gram $BaSO_4$ = 1·73 per cent. SO_3.
0·4705 gram substance† gave 0·0084 gram CuO = 1·78 per cent. CuO.
0·6011 gram substance gave 0·0107 gram CuO = 1·78 per cent. CuO.

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* Roasted and then ignited with pure sodium carbonate, the residue dissolved in hot water acidified with hydrochloric acid and precipitated with barium chloride.

† Ignited, the residue dissolved in nitric acid, precipitated with hydrogen sulphide, etc.

The molecular weight of CuO and SO_3 being the same, it is evident that the two are present in just the proportion to form cupric sulphate.

b. Iron compounds.

By adding a solution of ferric chloride to an ammonium chloride solution of myosin, a semi-gelatinous precipitate is formed of a reddish yellow color, and consisting of a combination of myosin and oxide of iron. The compound when thoroughly washed contains no chlorine. When freshly precipitated, it is partially soluble in dilute ammonium hydroxide, as also in sodium hydroxide, the residue becoming gummy or gelatinous and brownish yellow in color. It swells up in sodium carbonate, but is insoluble. In nitric acid the compound turns yellow, but is wholly insoluble and does not swell up. In hydrochloric and also in sulphuric acid the compound is likewise insoluble. In acetic acid, however, it is soluble completely, forming a semi-gelatinous fluid. In this, as in other metallic compounds of myosin, acids simply dissolve out the metal and then exert their usual action on the myosin. The various preparations, washed free from iron and chlorine, and dried at 110° C. were analyzed with the following results:

SERIES I.

No.	Amt. Sub. taken.	Wt. Fe_2O_3.	Per cent. Fe_2O_3.	Per cent. Fe.
1*a*	0·7046 gram.	0.0194 gram.	2·76	1·93
a	0·7021	0·0193	2·74	1·92
2*a*	0·3762	0·0128	3·40	2·38
b	0·3623	0·0121	3·33	2·33
3*a*	0·5837	0·0141	2·42	1·69
b	0·5618	0·0137	2·43	1·70
4*a*	0·5438	0·0177	3·25	2·26
b	0·6088	0·0197	3·24	2·26
5*a*	0·4761	0·0140	2·95	2·06
b	0·3381	0·0100	2·95	2·07
6*a*	0·5101	0·0173	3·40	2·37
b	0·5927	0·0194	3·28	2·29

SERIES II.

No.	Amt. Sub. taken.	Wt. Fe_2O_3.	Per cent. Fe_2O_3.	Per cent. Fe.
1	0·4847	0·0188	3·87	2·70
2	0·3200	0·0115	3·59	2·51
3	0·4118	0·0153	3·72	2·59
4	0.5205	0·0184	3·53	2·45

No.	Amt. Sub. taken.	Wt. Fe_2O_3.	Per cent. Fe_2O_3.	Per cent. Fe.
5*a*	0·4551 gram.	0·0167 gram.	3·68	2·57
b	0·3668	0·0137	3·73	2·61
6*a*	0·3783	0·0138	3·64	2·53
b	0·3675	0·0131	3·57	2·50
7*a*	0·4284	0·0143	3·34	2·33
b	0·4041	0·0136	3·37	2·36

SERIES III.

With a large excess of ferric chloride.

1*a*	0·4385	0·0269	6·13	4·29
b	0·6939	0·0436	6·27	4·38

In none of these preparations was there any attempt made to add a definite amount of ferric chloride, but the iron salt was added until a good precipitate was obtained. Undoubtedly, the amount of iron salt added, modifies materially the composition of the compound. In series III it is seen that the content of iron is about double the average amount contained in the other preparations. The average amount of iron (Fe) in the first two series of compounds is 2·29 per cent.

c. Zinc compounds.

With zinc sulphate, myosin is thrown down from its ammonium chloride solution as a heavy gelatinous precipitate. Like the iron compound it is partially soluble in sodium and ammonium hydroxides, swelling up to a gelatinous mass. It is insoluble in nitric, hydrochloric and sulphuric acids, but is partially soluble in acetic acid. In composition, it is seen to be very closely allied to the zinc albuminate. Following are the results obtained by analysis of the dried compounds:

SERIES I.

No.	Amt. Sub. taken.	Wt. ZnO.	Per cent. ZnO.	Per cent. Zn.
1*a*	0·6108 gram.	0·0049 gram.	0·81	0·64
b	0·6619	0·0055	0·83	0·66
2*a*	0·6611	0·0047	0·71	0·57
b	0·8006	0·0059	0·73	0·59
3*a*	0·4666	0·0046	0·99	0·79
b	0·4494	0·0044	0·99	0·79
4*a*	0·5936	0·0048	0·80	0·64
b	0·6926	0·0055	0·79	0·63

Series II.

No.	Amt. Sub. taken.	Wt. ZnO.	Per cent. ZnO.	Per cent. Zn.
1*a*	0·6258 gram.	0·0051 gram.	0·82	0·65
b	0·6618	0·0054	0·81	0·65
2*a*	0·5315	0·0061	1·21	0·97
b	0·5485	0·0065	1·18	0·91
3*a*	0·7925	0·0064	0·81	0·65
b	0·6913	0·0057	0·83	0·66
4*a*	0·4858	0·0062	1·27	1·02
b	0·5318	0·0066.	1·24	0·99
5*a*	0·6635	0·0056	0·85	0·68
b	0·6504	0·0050	0·76	0·61
6*a*	0·6263	0·0055	0·87	0·70
b	0·6639	0·0059	0·88	0·71

The average content of zinc (Zn) is 0·72 per cent. Unlike the iron and copper compounds, there is here less variation in the composition of the various preparations.

d. Nickel and cobalt compounds.

The extremely low percentage of zinc in the zinc-myosin compounds, as contrasted with the iron in the iron compounds, led us to make a nickel and cobalt preparation for the sake of comparison. The results, in both cases, accord more nearly with those of the iron compound, for although containing a higher percentage of metal than the latter, it was necessary to prepare them both under just such conditions as in the iron compound led to the highest percentage of iron, viz: a large excess of the precipitant. Following are the analytical results obtained with both substances:

With Ni $(NO_3)_2$.

No.	Amt. Sub. taken.	Wt. NiO_2.	Per cent. NiO_2.	Per cent. Ni.
1*a*	0·6165 gram.	0·0045 gram.	7·29	4·71
b	0·7016	0·0051	7·26	4·70

With CO $(NO_3)_2$.

1*a*	0·6479	0·0064	9·95 .	6·45
b	0·6696	0·0065	9·70	6·28
2*a*	0·6451	0·0056	8·75	5·67
b	0·6842	0·0060	8·84	5·72

e. Uranium compounds.

The addition of uranyl nitrate to an ammonium chloride solution of myosin produces a heavy gelatinous precipitate of a uranyl-myosin compound, which in solubility resembles the other myosin preparations. Washed free from excess of uranyl nitrate and from ammonium chloride and then dried at 110° C., the various preparations yielded on analysis the following results, the uranium being determined by simple ignition and weighing as uranoso-uranic oxide:

SERIES I.

No	Amt. Sub taken	Wt. U_3O_8.	Per cent U_3O_8	Per cent U.
1a	0·6373 gram.	0·0489 gram.	7·67	6·51
b	0·6836	0·0526	7·69	6·53
2a	0·7081	0·0592	8·36	7·09
b	0·7689	0·0655	8·51	7·22
3a	0·6418	0·0586	9·13	7·75
b	0·7809	0·0715	9·16	7·78
4a	0·6621	0·0541	8·17	6·93
b	0·7455	0·0608	8·15	6·91
5a	0·7525	0·0663	8·81	7·48
b	0·6964	0·0615	8·83	7·50
6a	0·7208	0·0590	8·18	6·94
b	0·7439	0·0607	8·15	6·91
7a	0·6648	0·0527	7·92	6·72
b	0·7101	0·0565	7·96	6·76

SERIES II.

No	Amt. Sub taken	Wt. U_3O_8.	Per cent U_3O_8	Per cent U.
1a	0·8736	0·0837	9·58	8·13
b	0·8570	0·0820	9·57	8·12
2a	0·5857	0·0593	10·12	8·59
b	0·7088	0·0715	10·09	8·56
3a	0·5715	0·0568	9·94	8·44
b	0·6958	0·0688	9·89	8·40
4a	0·6929	0.0556	8·02	6·82
b	0·7667	0·0615	8·02	6·82
5a	0·8031	0·0874	10·88	9·23
b	0·8272	0·0901	10·89	9·24
6a	0·7034	0·0553	7·86	6·67
b	0·6810	0·0540	7·92	6·72

These results show a variation in the content of uranium, amounting to nearly 3 per cent. (6·51–9·24 per cent.) Further, a comparison of the two series shows plainly that there is something in the nature of the second myosin solution, which tends to raise the content of uranium in the uranyl compounds; probably, the greater concentration or dilution of the solution. Evidently, then, the composition of the compound is, in part at least, determined by the conditions under which the uranyl salt and the myosin solution are brought together. The average amount of uranium contained in the preparations is 7·49 per cent.

f. Mercury compounds.

By adding a solution of mercuric chloride to an ammonium chloride solution of myosin, a heavy gelatinous precipitate is formed which soon changes to a flocculent one. Freed from the excess of mercury salt and ammonium chloride, the compound is found to be entirely free from chlorine. The substance is somewhat soluble in sodium hydroxide, swelling up first and then gradually dissolving. Dried at 110° C. and then analyzed, the following results were obtained. The mercury was determined as already described under mercury albuminate.

SERIES I.

No.	Amt. Sub. taken.	Wt. of Hg.	Per cent Hg.
1*a*	0·7609 gram.	0·0166 gram.	2·18
b	1·2911	0·0268	2·07
2*a*	1·2386	0·0270	2·17
b	0·9525	0·0198	2·07
3*a*	1·1856	0·0218	1·84
b	0·9261	0·0173	1·86
4*a*	1·3504	0·0257	1·90
b	0·9382	0·0178	1·90

SERIES II.

No.	Amt. Sub. taken.	Wt. of Hg.	Per cent Hg.
1	0·8917	0·0241	2·70
2	1·1196	0·0310	2·77
3*a*	0·9537	0·0271	2·84
b	0·8813	0·0259	2·93
4	0·9209	0·0270	2·93
5	0·8352	0·0241	2·89
6	0·8564	0·0217	2·53
7*a*	1·0696	0·0344	3·22
b	0·8550	0·0264	3·09

The average content of mercury (Hg) is 2·43 per cent. The results of each series show a fairly close agreement, but the two series do not compare with each other at all. Thus, the average amount of mercury in the compounds of the first series is 1·99 per cent., while in the second series the average amount rises to 2·87 per cent. This is another good illustration of the influence of the strength of the solution on the composition of the precipitate, and as in this case the presence of any ash could not interfere with the ultimate result, since the mercury was separated by distillation, it follows that the apparently higher content of mercury in the second series must be due to combination of the myosin with a larger amount of the metal. Further, it has been claimed* that in the case of the silver albuminate, it is possible under certain circumstances for the albuminate, when formed in a concentrated solution, to inclose a variable amount of albumin mechanically, and thus the apparent percentage of silver in the albuminate be reduced. If such was true of the myosin-mercury compounds, a far greater variation would be expected in the percentage of mercury in the different preparations of the same series.

The following table of comparisons shows the average content of metal in the albuminates formed from the two kinds of proteid matter.

		Egg-albumin.		Myosin.	
Copper compound,		0·94 per cent.	Cu	1·17 per cent.	Cu
Iron	"	0·95	Fe	2·29	Fe
Zinc	"	0·91	Zn	0·72	Zn
Uranyl	"	4·60	U	7·49	U
Mercury	"	2·89	Hg	2·48	Hg
Lead	"	2·56	Pb	....	....
Silver	"	4·09	Ag	....	....
Nickel	"			4·70	Ni
Cobalt	"			6·03	Co

Apparently, the two forms of albuminous matter, the albumin and globulin, do not form corresponding compounds with the metallic salts experimented with.

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* See Loew, Pflüger's Archiv für Physiologie, Band xxvi, p. 393.

XXI.—EGG-ALBUMIN AND ALBUMOSES. BY R. H. CHITTENDEN AND PERCY R. BOLTON, PH.B.

EVER since the albumose bodies were first separated from the products of fibrin digestion* with pepsin-hydrochloric acid, it has been our intention, already expressed, to subject the various individual albumins† to the action of purified pepsin under like conditions, and thus ultimately to acquire a comparative knowledge of the albumose bodies obtainable from these different sources. Already the albumose bodies from fibrin and the globuloses‡ have been subjected to a careful study and we present here the result of a study of the albumoses from egg-albumin.

In doing this, we have to report at the same time, the results of a study of the composition of egg-albumin itself. For we have made it a rule, in the series of experiments shortly to be described, to analyze a sample of each lot of albumin prepared for digestion. In this manner we have obtained data for a direct comparison of composition between the original sample of albumin and the products formed by its digestion. This we have deemed of considerable importance, for the data so obtained may throw considerable light on the nature of the changes involved in the formation of the albumoses; particularly, as to whether they are hydrolytic in their nature.

Four distinct samples of albumin were prepared, three of which were prepared in large quantities and served as material for the subsequent digestions.

Albumin A.

This was a preparation of coagulated egg-albumin, prepared especially with the view of obtaining a product wholly free from globulin. The method employed was essentially that recommended by Hammarsten.§ The whites of 120 eggs were freed from the yolks, then finely divided by shaking with glass, the fluid mixed with an equal volume of water or more, then shaken vigorously with air and finally filtered through cloth. The solution so obtained, was then saturated with crystals of magnesium sulphate at 20° C., for the complete removal of the globulin. The mixture was filtered through paper and the clear filtrate saturated with sodium sulphate. The precipitated albumin was then filtered and washed with a saturated solution of sodium sulphate, after which it was dissolved in water and dialyzed in running water until the magnesium and sodium sulphates were entirely removed. The fluid was then again filtered and the albumin finally coagulated by being poured into eight litres of boiling water, slightly acidified with acetic acid. The great bulk of the coagulum so obtained was at once placed in four litres of 0·1 per cent. hydrochloric acid, while a small sample for analysis was washed with 95 per cent. alcohol, finally with absolute alcohol and then dried, first at 100° C., and finally at 106° C., *in vacuo*, until of constant weight. The following table shows the results of the analysis of the product. The various determinations were made as described in the previous articles on these subjects, the sulphur being determined by fusion with potassium hydroxide and potassium nitrate in a silver crucible, according to the method designated by Hammarsten* as 1*a*.

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*W. Kühne and R. H Chittenden, Ueber Albumosen, Zeitschrift für Biologie, Band xx, p. 11.

†W. Kühne and R. H. Chittenden, Ueber die nächsten Spaltungsproducte der Eiweisskörper, Zeitschrift für Biologie, Band xix, p. 159.

‡W. Kuhne and R. H. Chittenden. Globulin und Globulosen, Zeitschrift fur Biologie, Band xxii, p. 409.

§See K. V. Starke, Beitrage zur Kenntniss des Serum- und Eialbumins, Jahresbericht für Thierchemie, 1881, p. 18.

Albumin B.

This albumin was prepared from the whites of 120 eggs by a somewhat different method. The albumin solution, after dilution with water, was made very distinctly acid with acetic acid, and the heavy precipitate of globulin, after it had well settled, removed by filtration. The acid fluid was then made exactly neutral with sodium carbonate and again filtered; it was then thymolized and dialyzed in running water for eight days. A little globulin, not precipitated by the acetic acid, was found in the bottom of the dialyzers when the salts had diffused out. This was filtered off and the perfectly clear fluid evaporated at 35–45° C., to perfect dryness. A sample of this preparation was ground fine, dried at 106° C. *in vacuo* and analyzed. It is perhaps questionable, whether all of the globulin is removed by this method. The precipitate with acetic acid was quite heavy, and as H. Dillner† has recently shown that the amount of globulin in egg-albumin, as

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* See Zeitschrift für physiolog. Chemie, Band ix, p. 289.

† Ueber die Globuline im Huhnereiweiss, Jahresbericht für Thierchemie, 1885, p. 31.

ANALYSIS OF ALBUMIN A.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with KOH + KNO_3. gram.	S %	Ash found gram.	Ash %
						c. c	T. ° C.	Pressure mm.					
I	0·3938	0·2455	6·92	0·7517	52.05	----	---	-----	-----	--- -	----	--- -	----
II	0·6406	0·4007	6·95	1·2247	51.98	----	---	-----	----	-----	----	-----	----
III	0·6133	-----	----	--- --	----	80·5	8·2	749·1	15·78	-----	---	-----	----
IV	0·6189	-- ---	----	-----	----	79·8	6·8	754·5	15·68	----	----	- - -	----
V	0·5209	------	----	------	----	66·6	6·8	766·9	15·81	-- -	----	-----	----
VI	0·5751	- --	----	------	----	----	---	-----	-----	0·0798	1·90	-----	----
VII	0·5926	------	----	------	----	----	---	-----	-----	0·0833	1·93	-----	-- -
VIII	0·6417	------	----	------	----	----	---	----	-----	0·0916	1 96	-----	---
IX	0·6366	-----	----	------	----	----	---	-----	-----	-----	- --	0·0023	0·37

Percentage composition of the ash-free substance.

									Average
C	52·24	52·18	----	----	----	----	----	----	*52·21*
H	6·95	6·98	----	----	----	----	----	----	*6·96*
N	----	----	15·83	15·74	15·85	----	----	----	*15·80*
S	---	----	----	----	----	1·91	1·94	1.97	*1·94*
O	-- -	----	---	----	----	---	----	----	*23·09*
									100·00

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	$BaSO_4$ after fusion with KOH + KNO_3. gram.	S %	Ash found. gram.	Ash %
I	0·5525	0·3447	6·93	1·0488	51·70	----	---	-----	----	------	----	-- ---	-
II	0·6350	0·3944	6·90	1·2070	51·83	----	----	--- -	----	------	-- -	-- --	----
III	0·4499	-----	----	-----	----	57·6	9·6	768·1	15·70	---- -	----	------	----
IV	0·3088	-----	----	-----	----	41·4	15·6	751 0	15·74	------	----	-----	----
V	0·6240	-----	----	-----	----	----	----	-----	----	0·0796	1·75	-----	----
VI	0·6166	-----	----	-----	----	----	----	-----	----	0·0856	1·90	--	----
VII	0·5464	-----	----	-----	----	----	----	--- -	----	0·0683	1·71	-----	---
VIII	0·5040	-----	----	-----	----	----	---	- ---	-- -	0·0670	1·82	------	----
IX	0·5690	-----	----	-----	----	----	----	-----	-- -	0·0751	1·81	-- --	----
X	0·4430	-----	----	-----	----	---	----	- -	----	------	---	0·0049	1·10
XI	0·4901	-- --	-- -	-----	----	----	----	-----	----	------	- -	0·0055	1·12

Percentage composition of the ash-free substance.

										Average
C	52·28	52·39	----	----	----	---	----	----	---	52·33
H	7·00	6·97	----	----	----	----	----	----	- -	6·98
N	----	----	15·87	15·91	----	---	---	----	----	15·89
S	----	----	----	----	1·78	1·93	1·74	1·85	1·84	1·83
O	----	----	----	----	----	- --	----	---	- -	22·97
										100·00

determined by the magnesium sulphate method, never reaches 1 per cent., but averages only 0·667 per cent., it seems probable that the greater portion is separated by the acetic acid. Further, Dillner has found that on the dialysis of a neutralized egg-albumin solution, the matter which separates out after a few days dialysis, is only in part globulin, but consists, in addition, of a somewhat insoluble body rich in sulphur. Hence, the substance which separated in our dialyzers, after precipitation with acetic acid and neutralization, may not have been composed wholly of globulin. The following table shows the composition of the uncoagulated albumin B.

Albumin C.

This preparation was much the same as albumin B, except that it was finally coagulated. Globulin was separated by acetic acid, the filtrate neutralized, again filtered and the fluid dialyzed in running water until all soluble salts were removed. The albumin was then coagulated by being poured into a large volume of boiling water acidified with acetic acid. A sample, after drying at 106° C. *in vacuo*, was found to have the composition shown in the accompanying table.

Albumin D.

This sample of albumin was prepared in exactly the same manner as albumin A; the globulin removed by magnesium sulphate, the albumin precipitated by sodium sulphate and after dialysis, coagulated as already described. Its composition is shown in the following table.

Comparing now, the results of the analysis of these four samples of albumin, it is seen that the first three agree almost exactly in composition, while the fourth shows a somewhat lower content of carbon.

	A	*B*	*C*	*D*	*Average.*
C	52·21	52·33	52·46	51·74	52·18
H	6·96	6·98	7·00	6·81	6·93
N	15·80	15·89	15·88	15·68	15·81
S	1·94	1·83	1·69	2·02	1·87
O	23·09	22·97	22·97	23·75	23·21
Ash	0·37	1·11	0·17	0·45	

Further, the coagulated products (A and C) do not differ at all in composition from the non-coagulated albumin B.

Schützenberger,* as a result of his work on proteid matter, ascribed

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* Bulletin de la Société Chimique de Paris, T. 23 et 24.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with KOH – KNO_3. gram.	S %	Ash found. gram.	Ash %
						c. c.	T °C.	Pressure mm.					
I	0·2930	0·1850	7·01	0·5633	52·42	----	----	- --	---	-----	----	----	----
II	0·6268	0·3933	6·97	1·2033	52·35	----	----	----	----	--- -	----	----	----
III	0·5936	-----	----	-----	----	79·5	15·7	752·4	15·74	-----	----	----	----
IV	0·3742	-----	----	-----	----	50·8	16·3	754·8	15·98	-----	----	----	----
V	0·5979	-----	----	-----	----	----	----	---	---	0·0736	1·69	----	----
VI	0·7139	-----	----	-----	----	----	----	----	----	0·0872	1·68	- --	----
VII	0·7333	-----	----	-----	----	---	----	----	----	--- -	----	0·0013	0·17

Percentage composition of the ash-free substance.

							Average
C	52·49	52·43	- --	- --	----	----	*52·46*
H	7·02	6·98	----	----	----	----	*7·00*
N	----	----	15·77	16·00	----	----	*15·88*
S	----	-- -	----	----	1·69	1·68	*1·69*
O	----	----	----	----	----	- --	*22·97*
							100·00

ANALYSIS OF ALBUMIN D.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. ° C.	Pressure mm.					
I	0·6009	0·3695	6·83	1·1340	51·46	----	----	-----	----	-----	----	-----	-----
II	0·4564	0·2765	6·73	0·8630	51·56	----	----	-- --	----	-----	----	-----	-----
III	0·5094	-----	----	-----	----	65·1	6·0	756·5	15·63	-----	----	-----	-----
IV	0·7652	-----	----	-----	----	96·0	4·7	765·5	15·60	--- -	----	--- -	- --
V	0·9402	-----	---	----	----	---	---	-----	----	0·1379	2·01	-----	-----
VI	0·6664	-----	----	----	----	----	----	- ---	----	0·0982	2·02	-----	-----
VII	1·2907	-----	-- -	-----	----	----	----	-----	--	- ---	---	0·0058	0·45
VIII	1·0975	----	----	----	----	----	----	-----	----	-----	----	0·0051	0·46

Percentage composition of the ash-free substance.

							Average.
C	51·69	51·79	----	----	----	----	*51·74*
H	6·86	6·76	----	----	----	----	*6·81*
N	----	----	15·69	15·67	----	----	*15·68*
S	----	----	----	----	2·02	2·03	*2·02*
O	---	----	----	----	----	----	*23·75*
							100·00

to albumin the formula $C_{240}H_{392}N_{61}O_{75}S_{3}$, which requires a content of carbon not far different from the average of our results, but which on the other hand demands a content of nitrogen nearly 1 per cent. higher than we found. The well-known Lieberkühn's formula requires 53·59 per cent. of carbon, or 1 per cent. more than was found in our highest result. Harnack's formula for albumin,* $C_{204}H_{322}N_{52}O_{66}S_{2}$, with a molecular weight of 4618, based on a study of the copper compounds of albumin, requires too high a content of carbon and altogether too low a percentage of sulphur. Lieberkühn's formula requires 1·98 per cent. of sulphur, while Harnack's formula requires only 1·39 per cent.; and as this was one of the main points on which Harnack based his formula, it is well to consider it. Our lowest result on sulphur is 1·69 per cent., and as the other three show a close agreement, it is probable that the former is somewhat too low. The average of our results, however, is but 0·04 per cent. higher than found by Lieberkühn. O. Loew† has recently considered this question, and he found on determining the sulphur in coagulated egg-albumin by a modification of Piria and Schiff's method, 1·70 and 1·87 per cent. of sulphur respectively. O. Nasse,‡ likewise, found in coagulated albumin a content of 1·72 per cent. of sulphur, and lastly, Hammarsten§ found in non-coagulated albumin 1·93 per cent. of sulphur. There would seem to be plenty of confirmatory evidence, therefore, that the content of sulphur in egg-albumin is much larger than indicated by Harnack's formula.

The nitrogen, as determined in our preparations, is seen to be somewhat higher than found by Hammarsten, with whose results in other respects ours most closely correspond. Dumas, however, found nearly the same percentage of nitrogen as contained in our preparations. The accompanying table of analyses shows the average of our results, compared with those of others.

Albumoses.

Three distinct digestions of albumin (preparations A, B and C) were made with pepsin-hydrochloric acid, and the albumose bodies isolated. In this way it was possible to prepare the bodies under somewhat different conditions, and to notice the influence, if any, on the nature and composition of the products. The pepsin-hydrochloric acid used in two of the digestions was prepared with a special view

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* Zeitschrift für Physiolog. Chemie, Band v, p. 207.

† Pflüger's Archiv für Physiologie, Band xxxi, p. 395.

‡ Jahresbericht für Thierchemie, 1873, p. 13. § Ibid, 1881, p. 19

ANALYSES OF EGG-ALBUMIN.

	1	2	3	4	5	6	7	8
C	53·5	53·51	53·37	52·25	52·18	53·59	52·58	53·01
H	7·0	7·03	7·10	6·90	6·93	6·95	7·15	6·98
N	15·5	15·61	15·77	15·25	15·81	15·65	16·61	15·76
S	1·6	1·83	-----	1·93	1·87	1·98	1·75	1·39
O	----	-----	-----	-- -	- -	21·83	21·91	22·86

1. As analyzed by Mulder. Journal fur prakt. Chemie. Band xliv, p 509.
2. As analyzed by Lieberkuhn. Poggendorff's Annalen 1852, p. 117.
3. As analyzed by Dumas and Cahours. Ann chem. Phys. (3) vi. p. 408
4. As analyzed by Hammarsten. Jahresbericht fur Thierchemie, 1881, p 19
5. Average of the results by Chittenden and Bolton
6. Theoretical composition according to Lieberkuhn's formula ($C_{[illegible]}H_{11[illegible]}N_{1[illegible]}SO_{22}$).
7. Theoretical composition according to Schutzenberger's formula ($C_{24[illegible]}H_{[illegible]2}N_{6[illegible]}O_{[illegible]}S_{[illegible]}$)
8. Theoretical composition according to Harnack's formula ($C_{[illegible]}H_{3[illegible]}N_{[illegible]2}O_{[illegible]}S_{[illegible]}$).

to removing all traces of albumose bodies, formed by the self-digestion of the mucous membrane, and was prepared as follows: 700 grams of mucous membrane from the cardiac portion of six pigs' stomachs, freed from the muscularis, were finely divided and warmed at 40° C. for fourteen days, in two and a half litres of 0·5 per cent. hydrochloric acid. At the end of this time, all albumose bodies presumably having been converted into peptone, the solution was filtered from the residue of nuclein, antialbumid, etc., and the filtrate saturated with ammonium sulphate. The precipitate, consisting mainly of pepsin, with perhaps some albumose, was filtered off, washed with a saturated solution of ammonium sulphate, and then dissolved in two litres of 0·2 per cent. hydrochloric acid. The acid solution was then thymolized and dialyzed in running water, until the ammonium sulphate was entirely removed. On opening the dialyzing tubes, quite a precipitate was found, which on being dissolved in 0·2 per cent. hydrochloric acid showed marked proteolytic action. The filtrate also, on being acidified, showed vigorous digestive power. These two solutions of purified pepsin were used in the digestion of two of the albumins, while with the third a pure glycerin extract of pepsin was employed.

The general method of procedure, both in the digestions themselves and in the separation of the various albumoses, was much the same as that previously employed by Kühne and Chittenden.

Digestion of Albumin A.

The albumin, as previously described, was placed in four litres of 0·1 per cent. hydrochloric acid and the mixture raised to a temperature of 45° C. Then 600 c. c. of the purified pepsin-hydrochloric acid solution were added and the mixture kept at a temperature of 45° C. for three hours, after which it was neutralized with sodium hydroxide and filtered. The pepsin solution, although quite active, did not act very vigorously on the coagulated albumin. The neutralization precipitate, therefore, together with the unaltered albumin, was again treated with a fresh quantity of the pepsin-hydrochloric acid, under like conditions as the preceding, for four hours. The two neutralized fluids were then united and treated together. The total volume was about six litres. The clear fluid was saturated in the cold with crystals of sodium chloride, by which a precipitate was obtained, which from analogy should consist of proto-, dys- and heteroalbumose.

In making these separations of the albumose bodies, we intentionally avoided raising the temperature of the fluid above 15° C., for fear that heat might induce some change in the character of the bodies; hence the first neutralized fluid was saturated directly with salt, in spite of its large volume, and the bodies were ultimately all separated without having been exposed to a temperature higher than that above-mentioned. The use, however, of such a large quantity of rock salt introduced into the solutions some calcium sulphate, which adhered very tenaciously to the albumose bodies and thus unavoidably raised the content of ash in the preparations.

The precipitate produced by the addition of sodium chloride in substance was filtered, washed with a saturated solution of sodium chloride, then extracted successively with a ten per cent. solution of sodium chloride, a five per cent. solution of the same salt, and lastly with water. The residue remaining undissolved after these successive treatments with dilute salt solutions and water, presumably consisted of dysalbumose, while the solutions contained a body precipitable by acetic acid and soluble in excess, and also precipitable by potassium ferrocyanide; presumably protoalbumose together with heteroalbumose. The original salt-saturated filtrate contained all of the deuteroalbumose, together with considerable protoalbumose and some heteroalbumose.

A. *Protoalbumose.*

The five and ten per cent. sodium chloride solutions of the first salt precipitate, together with the aqueous solution of the same, were united and then dialyzed in running water for removal of the heteroalbumose. The solution, partially freed from the latter, was concentrated somewhat and the protoalbumose again precipitated by saturating the solution with sodium chloride. This precipitate was again dissolved in water, dialyzed until the greater portion of the salt was removed, the solution then concentrated and the albumose precipitated by alcohol. This precipitate was redissolved in water, dialyzed until no chlorine reaction could be obtained with silver nitrate, the solution concentrated on the water bath to a syrup and finally precipitated with alcohol, washed with alcohol and ether and then dried at 106° C. *in vacuo* until of constant weight. In the last dialysis, there was no separation whatever of heteroalbumose, hence the protoalbumose is to be considered as quite pure. The composition of the substance is shown in the accompanying table. The ash contained no sulphate.

ANALYSIS OF A. PROTOALBUMOSE.

No	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with KOH – KNO_3, gram.	S %	Ash found. gram	Ash %
						c. c.	T C.	Pressure m m.					
I	0·4428	0·2699	6·77	0·8062	49·65	--	----	-- --	----	------	----	-----	-- -
II	0·2665	0·1607	6·70	0·4862	49·75	---	---	------	----	------		---	-
III	0·4825	- --	--	------	----	63·0	15·2	758·4	15·40	----	- --	--	----
IV	0·4038	------	---	---- -	----	52·1	14·3	758·2	15·36	- --	---	---	----
V	0·5392	------	---	------	----	----	----	- --	---	0·0673	1·71	----	- -
VI	0·5516	------	----	----	----	----	---	------	-	0·0733	1·80	- --	----
VII	0·6856	------	----	- --	- --	----	----	------	----	------	--	0·0172	2·50

Percentage composition of the ash-free substance.

							Average
C	50·92	51·03	----	- --	----		50·98
H	6·94	6·87	---	---	----	----	6·90
N	----	----	15·79	15·75	----	----	15·77
S	---	----	--	----	1·75	1·86	1·81
O	---	----	----	----	----	- -	24·54
							100·00

The protoalbumose was readily soluble in water and, unlike the protoalbumose from fibrin, dissolved to a perfectly clear solution with a neutral or very faintly alkaline reaction. The aqueous solution was rendered somewhat turbid by the addition of a little acetic acid, the turbidity disappearing, however, on the addition of an excess of acid. The aqueous solution, strongly acidified with acetic acid, was precipitated by the addition of potassium ferrocyanide; the precipitate, however, dissolved on heating the mixture, reappearing as the solution became cool.

An aqueous solution of the albumose, acidified with acetic acid to such an extent that the first turbidity was re-dissolved, was not rendered at all turbid by the addition of a little sodium chloride; the addition of more salt, however, gave a very strong turbidity which disappeared entirely on warming, reappearing on cooling. As with the protoalbumose from fibrin, it is possible to add such a quantity of sodium chloride as to induce a very heavy precipitate, yet have it wholly disappear on boiling the mixture, separating out again, however, as the solution becomes cool. Finally the addition of a larger amount of sodium chloride gave a precipitate in the acidified solution, which was not at all affected by even boiling.

An aqueous solution of protoalbumose, when treated drop by drop with concentrated nitric acid, was rendered noticeably turbid at the point of contact, the turbidity disappearing as the mixture was shaken. On adding just the right proportion of nitric acid, a point was reached where the solution showed a permanent turbidity, which disappeared on the application of a little heat, returning as the solution cooled. A slight excess of nitric acid produced even in the cold, a very distinct reddish yellow coloration of the fluid, the turbidity disappearing. By adding crystals of salt to the acid solution, a precipitate was again formed, which disappeared on the application of heat, and reappeared as the solution cooled.

By saturating an aqueous solution of protoalbumose with salt, a heavy precipitate was formed, but in the filtrate more albumose was always found on the addition of a little acetic acid. In fact, each time protoalbumose was precipitated by sodium chloride in substance there was always a loss; a certain proportion of the substance remaining in the filtrate, precipitable only by the addition of a little acetic acid. Protoalbumose heated with acid, or treated in the cold with dilute alkalies was not apparently converted into acid albumin or alkali-albuminate-like bodies, for on neutralization, no precipitation whatever occurred. Heated with potassium hydroxide and plumbic acetate, there was a decided blackening of the fluid. The protoalbumose likewise gave the characteristic reddish violet color with potassium hydroxide and cupric sulphate. Cupric sulphate alone, added to an aqueous solution of protoalbumose, gave a heavy greenish colored precipitate, not very soluble in excess of the copper salt. Mercuric chloride and lead acetate also precipitated the albumose.

In its reactions, therefore, the protoalbumose formed from egg-albumin does not differ, essentially at least, from fibrin protoalbumose.

A. *Deuteroalbumose.*

This body was obtained from the first salt-saturated fluid, by the addition of a little acetic acid (sp. gr. 1042) also saturated with salt. As Kuhne and Chittenden have already pointed out, all of the protoalbumose is not precipitated by saturation of a neutral fluid with sodium chloride. Hence, it is to be expected that the deuteroalbumose solution would contain some protoalbumose, which latter would be likewise precipitated by the salt-saturated acetic acid. We endeavored to make a separation, however, by rejecting altogether the first precipitate produced by the addition of a little acetic acid, and then to obtain the deuteroalbumose fairly free from the former, by the subsequent addition of more acetic acid. The final precipitate so obtained, was dissolved in a small amount of water and then dialyzed for several days. The solution, in which was noticed a small deposit of heteroalbumose, was concentrated and finally precipitated by alcohol. The precipitated deuteroalbumose was then redissolved in water, the solution made exactly neutral with sodium carbonate and dialyzed in running water for many days, after which the solution was concentrated to a syrup, the albumose precipitated with alcohol and finally, after washing with ether, dried at 106° C. *in vacuo* until of constant weight. The composition of the product is shown in the accompanying table. The ash was composed mainly of ferric oxide and calcium phosphate; it contained no sulphate.

The pure white powder, after being dried at 106° C, was found readily soluble in water. The solution was not rendered at all turbid by saturation with sodium chloride, but the substance was more or less completely precipitated by the addition of a little acetic acid to the salt-saturated fluid. Nitric acid added to an aqueous solution of the substance gave no precipitate whatever, but colored the solution decidedly yellow even in the cold. A little sodium chloride added to

ANALYSIS OF A. DEUTEROALBUMOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found gram.	Ash %
						c. c.	T. °C.	Pressure m.m.					
I	0·5091	0·3168	6·91	0 9596	51·40	----	---	---	----	------	---	------	----
II	0·4387	0·2725	6·90	0·8310	51·65	----	---	----	---	------	---	------	----
III	0·5222	-- --	----	------	----	69·5	15·8	753·5	15·66	- ---	-	------	----
IV	0·6229	------	----	------	---	80·5	14·0	761·6	15·47	------	----	------	----
V	0·4525	------	---	------	----	----	----	----	----	0·0566	1·71	----	-- -
VI	0.5961	------	----	------	----	---	----	----	----	0·0805	1·85	-----	- --
VII	0·6743	------	----	------	----	----	----	----	-	------	----	0·0078	1·15
VIII	0·5807	------	----	------	----	----	----	----	----	- ----	---	0·0059	1·01

Percentage composition of the ash-free substance.

							Average
C	51·96	52·19	-----	---	-----	-----	*52·07*
H	6·98	6·97	-----	--- -	----	-----	*6·97*
N	-----	--- -	15·83	15·64	- - -	----	*15·73*
S	----	-----	-----	-----	1·74	1·87	*1·80*
O	-----	-----	-----	-----	-----	-----	*23·43*
							100·00

the nitric acid solution gave a decided turbidity, which disappeared on warming the solution and reappeared on cooling.

The addition of acetic acid to an aqueous solution of the albumose gave no precipitate whatever, nor was any change to be observed on heating the fluid; neutralization, at least, caused no precipitation. The addition of a little sodium chloride solution to a solution of deuteroalbumose acidified slightly with acetic acid gave no precipitate whatever, but as with deuteroalbumose from fibrin, the application of a little heat induced a slight turbidity, which disappeared on raising the temperature still higher. Again, on the further addition of sodium chloride, a heavier precipitate was produced which disappeared completely on heating the solution and reappeared on cooling; and lastly, by adding more sodium chloride, a precipitate was obtained which was permanent even on heating the mixture to boiling. In these, as in nearly all other respects, the deuteroalbumose showed itself the same in nature as the deuteroalbumose from fibrin, and the reactions given for that body can well be applied here. In one reaction only was there any very noticeable difference; viz: in the reaction with cupric sulphate. Deuteroalbumose from egg-albumin gave only a slight precipitate with cupric sulphate, even on the addition of a minimum amount of the copper salt.* With acetic acid and potassium ferrocyanide, the reaction was much the same as with protoalbumose. Boiling with sodium hydroxide and lead acetate gave a decided blackening of the fluid, from the presence of sulphur.

A. *Heteroalbumose.*

The greater portion of the heteroalbumose was obtained by the dialysis of the 5 and 10 per cent. sodium chloride solutions of the first salt precipitate, viz: in the purification of protoalbumose. Some, too, was also found in the dialysis of the precipitated deuteroalbumose. In both cases, the albumose was left as a more or less gummy precipitate, closely adherent to the parchment of the dialyser, separating out as the sodium chloride left the solution. The product was purified by solution in 5 per cent. sodium chloride, re-precipitation by the addition of salt in substance, re-solution in 5 per cent. sodium chloride and separation by dialysis, continued until all chlorine was

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* This fact simply shows the greater purity of this preparation of deuteroalbumose or rather its freedom from protoalbumose, for as Dr. Neumeister has recently shown, perfectly pure deuteroalbumose gives no precipitate whatever with cupric sulphate. Later, we were able to prepare deuteroalbumose entirely free from protoalbumose.

ANALYSIS OF A. HETEROALBUMOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. C.	Pressure mm.					
I	0·4441	0·2720	6·80	0·8324	51·11	----	----	----	----	------	----	------	----
II	0·5365	0·3312	6·85	1·0047	51·07	----	----	----	----	------	----	----	----
III	0·3226	-----	----	------	----	42·1	15·6	754·1	15·39	------	----	------	---
IV	0·3255	-----	----	------	----	41·6	14·5	757·7	15·20	------	----	------	----
V	0·6451	-----	----	------	----	----	----	----	----	0·0751	1·59	------	----
VI	0·1772	-----	---	------	----	----	----	----	----	------	----	0·0033	1·86

Percentage composition of the ash-free substance.

						Average.
C	52·08	52·03	----	----	----	*52·06*
H	6·93	6·98	----	----	----	*6·95*
N	----	----	15·67	15·48	----	*15·55*
S	----	----	----	----	1·63	*1·63*
O	----	----	----	----	----	*23·81*
						100·00

removed from the solution. Like heteroalbumose from fibrin, this product each time it was re-dissolved in dilute sodium chloride, left a residue soluble only in dilute acids; presumably dysalbumose.

After being washed with water, alcohol and ether, the product was dried at 106° C. *in vacuo* and then analyzed with the results shown in the accompanying table.

The ash consisted mainly of calcium phosphate and a little ferric oxide, but did not contain any sulphate. The reactions of the body were found to be almost identical with those described as characteristic of heteroalbumose from fibrin.* Suspended in water or dissolved in 5–10 per cent. sodium chloride solution and then heated to boiling, the heteroalbumose was changed into a body, *coagulated heteroalbumose*, insoluble in sodium chloride but slowly soluble in 0·2 per cent. hydrochloric acid, from which it was precipitated by neutralization, apparently reconverted again, in part, into soluble heteroalbumose and in part into a body resembling dysalbumose. Thus on neutralizing the acid solution, a decided precipitate was obtained which was in part soluble in 5 per cent. sodium chloride (heteroalbumose), while the residue insoluble in the salt solution was soluble in dilute acids and in dilute alkalies (dysalbumose). In the filtrate from the neutralization precipitate, acetic acid showed the presence of still more heteroalbumose, which could be separated from the solution by dialysis.

Further, the heteroalbumose was found to be soluble in dilute acids, alkalies and alkaline carbonates, and from the solutions thus formed, it was reprecipitated by neutralization, but never completely; the amount remaining in solution being dependent naturally on the amount of neutral salt contained in the fluid.

Nitric acid precipitated the albumose, the extent of the precipitation depending on the amount of sodium chloride present. Acetic acid likewise gave a precipitate, soluble in excess of the acid. With acetic acid and potassium ferrocyanide, a precipitate was formed also soluble in excess of acid. With cupric sulphate, on the contrary, a heavy precipitate was obtained in a sodium chloride solution of the albumose, insoluble in excess of the copper salt. The substance gave the so-called biuret reaction with cupric sulphate and potassium hydroxide quite plainly, and also gave evidence of the presence of sulphur on boiling with potassium hydroxide and plumbic acetate.

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* See Zeitschrift für Biologie, Band xx, p. 32–36.

ANALYSIS OF B. PROTOALBUMOSE.

No.	Substance used. gram	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash. %	$BaSO_2$ from the ash. gram.	S of Ash % of Substance.	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	S after deducting S of Ash. %
						c. c.	T. °C.	Pressure mm.								
I	0·3540	0·1980	6·21	0·5784	44·55	----	----	------	----	----	---	------	------	--- --	----	--- -
II	0·4965	0·2709	6·06	0·8088	44·42	----	----	------	----	----	- --	------	------	-- --	----	-----
III	0·3794	------	----	------	----	45·5	17·8	755·2	14·05	----	- -	------	------	------	----	-----
IV	0·3771	------	----	------	----	45·1	18·1	762·8	14·13	----	----	------	------	------	----	-----
V	0·6098	------	----	------	----	----	----	------	----	0·0773	12·67	------	------	------	----	----
Vb	0·6098	-----	----	-----	----	----	----	------	----	----	- --	0·1018	2·29	------	----	-----
VI	0·7674	------	----	------	----	----	----	------	----	----	----	-----	- ----	0·2100	3·75	1·46

Percentage composition of the ash-free substance.

						Average.
C	51·00	50·88	----	----	- --	*50·94*
H	7·11	6·96	----	---	---	*7·03*
N	----	----	16·09	16·18	----	*16·13*
S	----	----	---	----	2·01	*2·01*
O	----	----	----	----	----	*23·89*
						100·00

Digestion of albumin B.

340 grams of the dry albumin, non-coagulated, were soaked in 2 litres of 0·4 per cent. hydrochloric acid for 24 hours, then warmed up to 45° C. and 1 litre of the purified pepsin-hydrochloric acid added, also warmed at the same temperature. The mixture was kept at 45° C. for 16 hours, then neutralized and filtered. The filtrate, containing the albumose bodies formed from the non-coagulated albumin, was then treated as already described under Albumin A.

B. Protoalbumose.

The protoalbumose isolated from this digestion, was purified in much the same manner as A protoalbumose, and did not differ from it in its reactions, except that with water it did not dissolve to quite so clear a solution; in fact its solution in water resembled more closely the aqueous solutions of protoalbumose from fibrin. During its final purification, it was dialyzed in running water until no chlorine reaction could be obtained with silver nitrate. In spite of this fact, however, the preparation contained a large percentage of ash, consisting mainly of calcium sulphate, ferric oxide and a little calcium phosphate.

The accompanying table shows the composition of the substance after drying at 106° C., *in vacuo*, until of constant weight.

In the purification of this protoalbumose, the substance was reprecipitated three times by saturating the aqueous solution of the precipitate with sodium chloride. By this treatment, as already stated, there is considerable loss, inasmuch as the precipitation of protoalbumose with salt in this manner is never complete, considerable remaining each time in the salt-saturated fluid. By adding a very little acetic acid, however, the protoalbumose is completely precipitated from the salt-saturated solution. The filtrates therefore, from the second and third precipitations of protoalbumose with salt alone were united, and the albumose remaining in them precipitated by the addition of a little acetic acid, saturated with sodium chloride. Our object was to see whether the protoalbumose which had at one time been precipitated by salt alone and then had finally become soluble in the salt-saturated fluid, differed at all in composition or in reaction from the protoalbumose still insoluble in the salt solution.

The albumose separated in this manner was purified by being dissolved in water, the solution made exactly neutral with sodium carbonate and dialyzed for several days. The fluid was then con-

ANALYSIS OF B. PROTOALBUMOSE.

Precipitated from NaCl solution, by CH_3–COOH.

No.	Substance used. gram	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash. %	$BaSO_4$ from the ash. gram.	S of Ash. % of Substance.	$BaSO_4$ after fusion with KOH + KNO_3. gram.	S %	S after deducting S of Ash. %
I	0·5802	0·3495	6·69	1·0523	49·46	----	---	------	----	----	----	--- --	------	--- --	--	-----
II	0·3114	------	----	------	----	40·8	19·0	765·2	15·49	----	----	------	-----	-----	----	-----
III	0·4942	------	----	------	----	---	----	-----	----	0·0142	2·87	------	------	--- --	----	-----
III*b*	0·4942	---- -	----	------	---	---	----	------	----	----	----	0·0091	0·25	------	----	-----
IV	0·5084	------	----	-- --	---	----	----	------	----	-- -	----	- - --	------	0·0800	2·16	1·91

Percentage composition of the ash-free substance.

				Average.
C	50·91	----	----	*50·91*
H	6·89	----	----	*6·89*
N	---	15·94	-	*15·94*
S	---	--	1·98	*1·98*
O	----	----	-- -	*24·28*
				100·00

centrated to a syrup and the albumose precipitated by alcohol. This precipitate was again dissolved in water, the solution made exactly neutral and again dialyzed.

After suitable concentration, the albumose was again precipitated by alcohol, washed with alcohol and ether and finally dried at 100° C. *in vacuo.*

The results of the analysis are seen in the accompanying table.

The ash in this preparation is seen to be much smaller than in the protoalbumose precipitated by salt alone. In other respects the two analyses are closely comparable, particularly the carbon and sulphur.

The reactions were in almost every case the same as with the preceding preparation, excepting perhaps a somewhat greater solubility.

B. *Deuteroalbumose.*

This body was separated and purified in exactly the same manner as in the preceding digestion. The analysis of the product is shown in the accompanying table. The ash contained some calcium sulphate and a little ferric oxide. The reactions of the body were the same as those of A deuteroalbumose.

From this digestion, more or less heteroalbumose was separated but no analysis was made of the product, as the amount was rather small for the necessary purification.

Digestion of Albumin C.

In the digestion of this sample of coagulated albumin, a much more vigorous pepsin-hydrochloric acid was employed than in the preceding digestions. The freshly coagulated albumin was placed in 3 litres of 0·4 per cent. hydrochloric acid and brought to a temperature of 45° C., then 400 c. c. of a pepsin solution, made from a pure glycerin extract of pepsin, were added and the mixture kept at 45° C. for 24 hours. The fluid was then neutralized, filtered and the clear filtrate saturated with sodium chloride. The albumose bodies were then separated and purified according to the methods already described.

The several bodies showed the same reactions as observed in the preceding preparations.

Protoalbumose and deuteroalbumose were analyzed. The results are shown in the accompanying tables. The ash of the deuteroalbumose contained no sulphate, but was composed almost entirely of ferric oxide.

ANALYSIS OF B. DEUTEROALBUMOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %	$BaSO_4$ from the Ash. gram.	S of Ash % of Substance.	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	S after deducting S of Ash. %
						c. c.	T. °C.	Pressure m.m.								
I	0.3821	0·2359	6·86	0·7065	50·42	----	----	------	----	----	----	------	------	------	----	-----
II	0·2411	0·1480	6·82	0·4453	50·37	----	----	------	----	----	----	------	------	------	----	-----
III	0·4945	------	----	------	---	65·2	18·6	760·1	15·50	----	---	------	-----	------	----	----
IV	0·5393	------	----	------	----	71·5	19·4	760·2	15.55	----	----	-----	------	------	----	----
V	0·5424	------	----	------	----	----	----	------	----	0·0085	1·56	------	------	------	---	-----
Vb	0·5424	------	----	------	----	----	----	------	----	----	---	0·0055	0·09	------	----	-
VI	0·5292	------	--	-----	----	----	---	------	----	----	----	------	------	0·0820	2·12	2·03
VII	0·3578	-----	----	-----	----	----	---	------	----	----	----	------	------	0·0550	2·11	2·02

Percentage composition of the ash-free substance.

							Average.
C	51·21	51·16	----	----	----	----	*51·19*
H	6·96	6·92	----	----	----	----	*6·94*
N	----	----	15·75	15·80	----	----	*15·77*
S	----	----	----	----	2·03	2·02	*2·02*
O	----	----	----	----	--	----	*24·08*
							100·00

Dysalbumose.

This form of albumose was found in all three digestions, but the amount was much smaller than noticed in the fibrin digestions. The substance was obtained as an insoluble residue, after extracting the first sodium chloride precipitate successively with 10 and 5 per cent. salt solutions and with water. It was then dissolved in 0·2 per cent. hydrochloric acid, and after filtration precipitated by neutralization. After precipitation in this manner, a portion of the substance was found soluble in sodium chloride and on dialysis of the solution, separated in much the same manner as heteroalbumose. The portion still insoluble in salt solution was then washed thoroughly with water and lastly with alcohol and ether. Not enough of the albumose was separated from any one digestion for analysis, but by uniting the products from all three, sufficient was obtained for the following analytical data:

I. 0·2537 gram substance gave 0·1500 gram H_2O=6·57 per cent. H and 0·4550 gram CO_2=48·92 per cent. C.

II. 0·3700 gram substance gave 46·3 c. c. N at 13·8° C. and 761·3^{mm} pressure=14·96 per cent. N.

III. 0·1354 gram substance gave 0·0069 gram ash=5·09 per cent.

The ash-free substance therefore contained 51·52 per cent. C, 6·92 per cent. H, 15·79 per cent. N. The ash was composed wholly of ferric oxide.

The peculiar behavior of dysalbumose after solution in either dilute acids or sodium carbonate and neutralization, shows plainly that the substance is simply heteroalbumose rendered insoluble by action of the sodium chloride. Dysalbumose, wholly insoluble in sodium chloride, is readily dissolved by sodium carbonate of 1 per cent., and on neutralization of the alkaline fluid is in great part precipitated. The substance however, is now soluble in sodium chloride and has evidently been reconverted into heteroalbumose. It is very apparent, however, from our results that heteroalbumose from egg-albumin is not so readily converted into dysalbumose by the action of sodium chloride, as heteroalbumose from fibrin. In all three of our experiments, the amount found was very small.

Further, it would seem as if heteroalbumose from albumin was somewhat more resistant to the action of alcohol and ether than heteroalbumose from fibrin. Still the former did become quite rapidly insoluble in sodium chloride after standing under alcohol

ANALYSIS OF C. PROTOALBUMOSE.

Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram	Ash %	$BaSO_4$ from the Ash gram.	S of Ash % of Substance.	$BaSO_4$ after fusion with $KOH + KNO_3$. gram	S %	S after deducting S of Ash %
					c. c	T. °C.	Pressure m.m.								
0·3618	0·2087	6·41	0·6097	45·95	---	----	------	----	----	----	------	------	-----	----	-----
0·4489	0·2547	6·30	0·7582	46·05	---	----	------	----	----	----	------	- ---	------	----	-----
0·3283	------	---	------	----	40·9	17·6	748·1	14·47	----	----	------	------	--- --	----	-----
0·3028	------	----	------	----	----	----	- ----	----	0·0321	10·60	-----	------	--- --	----	-----
0·3028	------	----	------	-- -	----	---	------	----	----	----	0·0500	2·26	------	----	- -
0·3962	------	----	---- -	---	----	----	-- ---	----	----	----	------	-----	0·1100	3·81	1·55

Percentage composition of the ash-free substance.

					Average.
C	51·59	51.48	----	----	51·44
H	7·16	7·05	----	----	7·10
N	----	---	16·18	----	16·18
S	---	----	----	2·00	2·00
O	----	----	----	----	23·28
					100·00

ANALYSIS OF C. DEUTEROALBUMOSE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. °C.	Pressure m.m.					
I	0·4262	0·2682	6·99	0·7978	51·05	----	----	------	----	------	----	------	---
II	0·3394	0·2104	6·88	0·6326	50·83	---	----	------	----	----	----	---- -	----
III	0·4465	------	----	------	----	59·9	18·7	760·1	15·77	----	----	-----	----
IV	0·3451	------	----	-----	----	46·8	20·4	756·6	15·77	-- ---	----	-----	- -
V	0·4508	------	----	------	----	----	----	--- --	----	0·0680	2·07	------	----
VI	0·4715	------	---	------	----	----	----	- --	---	0·0716	2·08	-- --	----
VII	0·4769	------	----	------	----	----	----	------	- -	- ---	----	0·0061	1·28

Percentage composition of the ash-free substance.

							Average.
C	51·70	51·49	----	----	----	----	*51·60*
H	7·08	6·97	----	----	----	----	*7·02*
N	---	----	15·98	15·98	----	----	*15·98*
S	----	----	----	----	2·08	2·09	*2·08*
O	----	----	----	----	----	----	*23·32*
							100·00

for a short time. The reactions of dysalbumose, aside from its behavior towards sodium chloride, were found to be much the same as those of heteroalbumose.

Relation of the albumoses to albumin.

In composition, the albumoses from albumin are seen to differ from each other somewhat more than the albumoses from fibrin; collectively, however, there is less difference in composition between the albumose bodies and the albumin from which they are formed, than noticed in the case of the albumose bodies from fibrin.* In the latter, however, there is no guarantee that the fibrin employed in the experiments had the actual composition assigned to pure blood-fibrin. The fibrin-albumoses collectively contained about 50·6 per cent. of carbon and 17·1 per cent. of nitrogen, while Hammarsten found fibrin itself to contain 52·6 per cent. of carbon and 16·9 per cent. of nitrogen. In our experiments, on the other hand, we have for comparison the composition of the albumin actually used in the experiments, and in the accompanying table the differences in composition of the various products are plainly to be seen.

Examining these in detail, we see that all of the products show a somewhat smaller content of carbon than albumin itself. With nitrogen, however, there is a very close agreement throughout, and with sulphur likewise. In the case of the fibrin-albumoses it was considered that the diminished percentage of carbon indicated plainly that the albumoses were hydration products, and that they were formed from fibrin by simple hydrolytic action. The results obtained with the globuloses did not appear to confirm this view, but in this case it must be remembered that the digestion of globulin by gastric juice may be quite a different process from albumin digestion. With albumin, however, the results, although less pronounced, also indicate hydrolytic action and that the products formed are hydration products.

The following table shows the extent of these differences, and also shows the close agreement in composition between proto- and deutero-albumose and the so-called soluble and insoluble hemialbumose from egg-albumin, isolated and analyzed by Kühne and Chittenden at the commencement of their study of these bodies.*

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* See Zeitschrift fur Biologie, Band xix, p. 171.

	Protoalbumose.				Deuteroalbumose			Hetero-albumose	Dys-albumose	Albumin
	A	B*	B†	C	A	B	C	A	A, B & C	Average of A, B & C
C	50·98	50·94	50·91	51·44	52·07	51·19	51·60	52·06	51·52	52·33
H	6·90	7·03	6·89	7·10	6·97	6·94	7·02	6·95	6·92	6·98
N	15·77	16·13	15·94	16·18	15·73	15·77	15·98	15·55	15·79	15·85
S	1·81	2·01	1·98	2·00	1·80	2·02	2·08	1·63		1·82
O	24·54	23·89	24·28	23·28	23·43	24·08	23·32	23·81		23·02
Ash	2·50	12·67	2·87	10·60	1·08	1·56	1·28	1.86	5.09	0·55

* Sodium chloride precipitate.

† Acetic acid precipitate

	Fibrin products.			Egg-albumin products.				
	Proto-albumose †	Deutero-albumose †	Fibrin.‡	Soluble Hemi-albumose *	Insoluble Hemi-albumose *	Protoalbumose §	Deuteroalbumose §	Egg-albumin
C	50·77	50·65	52·68	50·89	51·01	51·07	51·62	52·33
H	6·78	6·83	6·83	6·81	6·89	6·98	6·97	6·98
N	17·14	17·17	16·91	15·98	15·79	16·00	15·82	15·85
S	1·08	0·97	1·10			1·95	1·96	1·82
O	24·23	24·38	22·48	.	.	24·00	23·63	23·02

Deuteroalbumose is seen to contain 0·7 per cent. less carbon than egg-albumin, while protoalbumose contains fully 1·25 per cent. less. The nitrogen in the two compounds is in accord with the content of carbon and the composition of the two products certainly suggests hydrolytic action.

The lower content of carbon in the albumose bodies has been explained by some writers on the ground that the precipitants used, principally acetic acid, would tend to form an acid compound, and that even when dried and ready for analysis, the compound would still contain some acid; which fact would be sufficient to account for the low content of carbon found. It is to be noticed, however, in these results that the deuteroalbumose, not only on an average but in each individual case, contains more carbon than protoalbumose, which was precipitated by sodium chloride alone without the addition of any acetic acid whatever. Further, comparing the two preparations of protoalbumose B, one of which was precipitated by salt alone and the other by acetic acid, it is seen that the percentage of carbon is the same in both. Unquestionably proto- and deuteroalbumose both do combine with acids, but after neutralization and long continued dialysis the albumose body certainly exists in a free state, uncombined with either acid or alkali.

The greater portion of this work was completed before we had any knowledge of Dr. Neumeister's work on the complete separation of protoalbumose from deuteroalbumose.‖ It was, therefore, too late for

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* See Kühne and Chittenden, Ueber die nächsten Spaltungsproducte der Eiweisskorper. Zeitschrift für Biologie, Band xix, p. 171.

† Average of all the products analyzed. Zeitschrift fur Biologie, Band xx, p. 40.

‡ According to Hammarsten. § Average of all products.

‖ Zur Kenntniss der Albumosen. Zeitschrift fur Biologie, Band xxiii, p. 381.

us to take advantage of his method of separation. Our preparations of deuteroalbumose unquestionably were not wholly free from protoalbumose, but that they did not contain much of this body is evidenced by the small precipitate obtained with cupric sulphate; for, as Dr. Neumeister has recently shown, deuteroalbumose entirely free from protoalbumose gives no precipitate whatever with cupric sulphate.

XXII.—Casein and its Primary Cleavage Products. By R. H. Chittenden and H. M. Painter, B.A., Ph.B.

Following out the general plan of procedure indicated some time ago with other albuminous bodies,* we have endeavored to prepare and study the primary products formed in the digestion of pure casein with pepsin-hydrochloric acid. Assuming that casein in its conversion into peptone by artificial gastric juice, passes through certain intermediate stages, in which bodies akin to the albumose bodies are formed, we have applied the methods of separation used so successfully in the past and have been able to isolate a class of bodies bearing the same relationship to casein that the albumoses do to albumin. For this class of bodies we propose the name of *caseoses.*

In studying these substances and particularly their composition, we deemed it essential to be certain of the purity and composition of the casein to be digested; particularly in view of the recent controversy between Hammarsten† and A. Danilewsky as to the single nature of this albuminous body. Assuming, as claimed by Danilewsky,‡ that casein is a mixture of two albuminous bodies and that the numerous analyses recently made by Hammarsten of various preparations of casein are incorrect, particularly in the percentage of sulphur, made it incumbent on us to obtain some data on these points confirmatory of one or the other view, before advancing to a study of the products formed by the digestion of casein.

Of the various methods used for the preparation of pure casein, that depending on repeated precipitation by acids and re-solution in alkalies has been the most in vogue; for although theoretical objections might be advanced as to the possibility of change in the nature of the substance under the influence of acids and alkalies, the results obtained have in some respects, at least, been very satisfactory. Alex. Schmidt, in conjunction with Kapeller,§ showed plainly that dialysis of milk and then precipitation of the casein by acetic acid, while it gave fairly good results, was not sufficient in itself to wholly remove the inorganic salts, and thus recourse was had to repeated precipitation by acids, after solution in dilute alkalies. By this method, Hammarsten* came to the conclusion that milk contains but two albuminous bodies, viz: casein and lacto-albumin, and the same investigator has repeatedly made use of this method for the preparation of pure casein. Lundberg† has plainly shown the noticeable resistance of casein to the action of acids, and Hammarsten has indicated the possibility of using acetic acid, if necessary, in place of the stronger hydrochloric acid. Millon and Commaille, however, have claimed that in the precipitation of casein with either acid, the precipitate does not consist of free casein, but is a compound of casein with the acid used.

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* See the preceding articles on globuloses and albumoses.

† See Zeitschrift fur physiologische Chemie. Band vii, p. 227. Zur Frage, ob das casein ein einheitlicher stoff sei. Also same volume, p. 427.

‡ See Zeitschrift fur physiologische chemie, Band vii, p. 433.

§ Beitrag zur Kenntniss der Milch, Jahresbericht für Thierchemie, 1874, p. 154

This erroneous view, Hammarsten shows depends simply on the great difficulty of washing the precipitated casein completely, and he suggests that it is perhaps impossible to prepare large quantities of casein absolutely pure. For the preparation of the substance, however, Hammarsten recommends acetic acid in preference to hydrochloric acid and final drying of the compound at a temperature of 110° C.

Danilewsky and Radenhausen,‡ however, prefer to use hydrochloric acid in the preparation of pure casein, and for this purpose they use skimmed milk, diluted with 4–5 volumes of water, to which the dilute acid is added little by little, until a good precipitate is obtained. After filtration and repeated washing with distilled water, the casein is rubbed fine, then dissolved in water to which a little ammonia has been added, the fluid filtered and the clear filtrate again precipitated by the addition of a little dilute hydrochloric acid. Casein, so prepared, after being washed with distilled water, reacts acid to test papers and shows the usual reactions of this body; but if when freshly precipitated, the substance is boiled with perfectly neutral 50 per cent. alcohol and filtered hot, according to Danilewsky and Radenhausen, the casein is separated into two bodies, one of which is partially soluble in hot alcohol and separates out on cooling, while the other is insoluble. The soluble portion is termed caseoprotalbin, the insoluble portion caseoalbumin. The former, it is stated, gives no sulphur reaction when boiled with 2 per cent. sodium hydroxide, but contains 1·13 per cent. of sulphur, while caseoalbumin is stated to contain 1·23 per cent. of sulphur. Hence, according to these investigators, casein is a mixture of two bodies, one of which is rich in sulphur, while the other contains a somewhat smaller amount. The objection which these investigators make to the use of acetic acid in the preparation of casein, is that from a sodium acetate solution, casein itself is not precipitated, or only in part, but that the precipitate consists mainly of the protalbin body. Further, Danilewsky and Radenhausen claim that caseoalbumin dissolved in 1 per cent. sodium hydroxide and allowed to stand for 24 hours at the temperature of the room, is changed almost completely into caseoprotalbin, with loss of sulphur and calcium phosphate. In a similar manner, protalbin dissolved in lime water, with addition of alcohol and phosphoric acid, can be changed into caseoalbumin.

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* Zur Kenntniss des Caseins und der Wirkung des Labfermentes, Jahresbericht für Thierchemie, 1877, p. 158.

† Kleinere Beiträge zur Kenntniss des Caseins, Jahresbericht für Thierchemie, 1876, p. 11.

‡ Untersuchungen über die Eiweissstoffe der Milch, Jahresbericht für Thierchemie, 1880, p. 186.

Hammarsten,* however, takes exception to these views and points out that the peculiar behavior of Danilewsky's casein towards boiling 50 per cent. alcohol, depends in part upon its content of calcium phosphate, the presence of which impurity depends upon the use of hydrochloric acid in the precipitation of the casein, which acid does not favor the removal of the salt as well as acetic acid. Using acetic acid as the precipitant and then employing a casein three times so precipitated, and which analysis showed to be almost entirely free from calcium phosphate, Hammarsten, by treatment with boiling alcohol, was unable to obtain anything more than a trace of substance corresponding to caseoalbumin. Further, casein is unquestionably changed by boiling with alcohol, as Hammarsten clearly shows; in fact it is well known that heating an albuminous body in water is liable to change its nature, at least its solubility, and there is no reason why treatment with 50 per cent. alcohol should not lead to a like result. Again, Hammarsten points out clearly another inconsistency in the reasoning of Danilewsky and Radenhausen in connection with the so-called conversion of caseoprotalbin into caseoalbumin. The former body is stated to be poorer in sulphur than the latter, and yet we are told that the protalbin body can be converted into caseoalbumin by simple solution in lime water and addition of phosphoric acid, with or without alcohol. Yet how it is possible by this method of treatment to convert a body with a small content of sulphur into a body richer in sulphur, is hard to see. Much more plausible is it, as suggested by Hammarsten, that in these two bodies we have to deal with the same substance, in the one case united with calcium phosphate, and in the other uncombined with this salt; or in other words that the so-called protalbin body in the absence of calcium phosphate is soluble in boiling 50 per cent. alcohol, while in the presence of that salt it is insoluble.

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* Zur Frage, ob das Casein ein einheitlicher Stoff sei. Zeitschrift fur physiologische chemie, Band vii, p. 227.

This view being correct, and Hammarsten's observations would tend to show that it is, it is obvious that the caseoprotalbin of Danilewsky is simply a portion of the casein, which, owing to lack of a sufficient amount of calcium phosphate, passes into solution on being boiled with dilute alcohol; while caseoalbumin, on the other hand, is likewise a portion of the casein, *insoluble* on account of the presence of calcium phosphate; changed, however, more or less by action of the boiling alcohol. Further, the reason why casein precipitated several times by acetic acid does not contain as much calcium phosphate as when precipitated by hydrochloric acid, and thus reacts differently with alcohol, depends on the far greater insolubility of freshly precipitated casein in excess of acetic acid than in hydrochloric acid. In the precipitation of casein with hydrochloric acid only the slightest excess of acid can be added, on account of the ready solubility of the precipitate in this dilute acid. With acetic acid, however, a moderate excess can be added without solution of the precipitate, and thus in the latter case, a larger proportion of mineral salts are removed at each re-precipitation.

Danilewsky and Radenhausen have further called attention to the fact that casein precipitated with hydrochloric acid yields a larger amount of alkaline sulphide than when precipitated by acetic acid. This statement, Hammarsten has several times been able to verify, but the latter investigator seeks an explanation for this fact in the occasional presence of a second albuminous body, richer in sulphur, presumably serum-globulin, precipitable like casein by acids. Serum-globulin too, is readily soluble in excess of acid, even more so than casein, and hence by the acetic acid method of precipitation, which allows a far greater excess of acid, the casein would be much less liable to contamination by this hypothetical globulin than by the hydrochloric acid method. In this connection it may be well to notice that Musso and Menozzi* claim the presence in milk of a peculiar albuminous body containing 53·74 per cent. C, 15·52 per cent. N and 1·55 per cent. S, for which they claim a position midway between serum-albumin and casein. It can be partially precipitated from fresh milk at ordinary temperatures by the addition of acetic acid. Further, Lebelien* has proved the presence in milk of a globulin-like body, lacto-globulin, which can be precipitated by saturating the fluid remaining after removal of the casein with sodium chloride, with magnesium sulphate. The substance appears to be identical with paraglobulin, and thus this fact, just discovered, would seem to confirm Hammarsten's theory as to the cause of the greater content of sulphur sometimes noticed in casein precipitated by hydrochloric acid.

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* Studien uber das Eiweiss der Milch. Jahresbericht fur Thierchemie, 1878, p. 139.

Accepting then, Hammarsten's views as correct, it is obvious that casein precipitated by acetic acid, if not a single body, must be composed of two bodies, more or less alike and both precipitable by dilute acids. In attempting to settle this point definitely, Hammarsten has sought by analysis of a large number of preparations made under different conditions, to obtain data as to the exact composition of casein variously prepared. Naturally in this connection, considerable attention was paid to the content of sulphur, since, Danilewsky and Radenhausen's views being correct, variations in the content of sulphur would naturally be expected. If casein is a mixture of equal parts of caseoprotalbin and caseoalbumin with 1·13 per cent. and 1·23 per cent. of sulphur, respectively, then casein itself would naturally contain 1·18 per cent. of sulphur; an amount somewhat higher than has been found heretofore.

Recently, Dainlewsky† has modified his views somewhat, and now considers casein, as before, to be a mixture of two distinct bodies, but of nucleoalbumin with nucleoprotalbin instead of caseoalbumin and caseoprotalbin. As the reactions of these two bodies are apparently much the same as those given as characteristic of the caseo-bodies, this change of view appears to be mainly a change of name. Danilewsky still claims the correctness of the high content of sulphur in casein and assumes that the variation in the results obtained by different workers is due simply to difference in the methods of determination, and that unquestionably pure casein contains over 1·0 per cent. of sulphur.

The content of sulphur in casein as determined 30–40 years ago by

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* Beitrag zur Kenntniss der Eiweisskorper der Kuhmilch. Jahresbericht fur Thierchemie, 1885, p. 184.

† See Zeitschrift fur physiologische chemie, Band vii, p. 433.

Lehmann, Rühling, Völckel and others,* varies from 0·85 per cent. to 1·10 per cent. Ritthausen,† from several analyses of the copper compound of casein, found a content of sulphur equivalent to 0·80–1·12 per cent. in the free casein. Schwarzenbach,‡ by a study of the platinum cyanide compound of casein, ascribed to casein itself a content of 0·19–1·10 per cent. of sulphur. Hammarsten, however, found by analysis of eight distinct preparations of casein, some of which had been reprecipitated even ten times with acetic acid, a content of sulphur ranging from 0·619 per cent. to 0·775 per cent.§ Later, Hammarsten‖ analyzed four other preparations of casein and each by six distinct methods. Omitting two or three results, which were altogether too low on account of inaccuracies in the method, Hammarsten found in these different preparations of casein, as a result of twenty-nine distinct determinations by five different methods, 0·798 per cent. as maximum, 0·726 per cent. as minimum, or 0·758 per cent. as the average, content of sulphur. Taking, however, the results obtained by what Hammarsten considers as the more correct methods the average content of sulphur is raised to 0·77–0·78 per cent. In no case did Hammarsten obtain results in any way confirmatory of Danilewsky's views. Hammarsten further made a large number of phosphorus determinations, and these as well as the results obtained for carbon and nitrogen showed too little variation to warrant the idea of a mixture of two bodies of unlike composition. While therefore, Hammarsten's results would seem to point conclusively to the unit-like nature of casein, we have, however, made quite a number of different preparations of the substance, both from fresh milk and from skimmed milk, with the idea of obtaining confirmatory data, with which to make direct comparisons between the composition of casein and its primary cleavage products.

Preparation and composition of Casein.

The casein was precipitated in some cases by acetic and in others by hydrochloric acid. In both cases the acid used was very dilute, the hydrochloric acid being 0·2 per cent. In dissolving the casein for re-precipitation, a very dilute solution of ammonium hydroxide was employed; in fact so dilute as to consist of hardly more than water with a trace of ammonia. We used ammonia, in preference to sodium or potassium hydroxide, as this alkali would seem less liable to induce any alteration in the content of sulphur. Further, in dissolving the casein in ammonia, the solution at no time became more than very faintly, if at all alkaline; usually being hardly more than neutral to test papers.

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* See Gmelin-Krauts' Handbuch der Organische Chemie, Band iv, Abtheilung, iii, 1870, p. 2254.

† H. Ritthausen und R. Pott, Untersuchungen über Verbindungen der Eiweisskörper mit Kupferoxyd. Journal für prakt. Chemie, 1873. Band vii, p. 361.

‡ Annalen der Chem. u. Pharmacie, Band cxxxiii, p. 185.

§ Zeitschrift fur physiologische Chemie, Band vii, p. 259.

‖ Ueber den Gehalt des caseins an Schwefel und über die Bestimmung des Schwefels in Proteinsubstanzen. Zeitschrift fur physiologische chemie, Band ix, p. 273.

The general method of procedure was to dilute fresh cow's milk with about four volumes of water (skimmed milk diluted considerably less) and then to precipitate the casein with either hydrochloric or acetic acids, adding the precipitant cautiously, until complete precipitation was obtained. The precipitate was then washed as completely as possible with large quantities of water, both by decantation, trituration with water in a mortar and on a cloth filter. The casein was then dissolved in the ammonia water, filtered through paper and reprecipitated, each time being thoroughly washed with water. In the portion used for analysis, the final precipitate was further washed with alcohol and ether and lastly soaked in a mixture of alcohol and ether for the more complete removal of any fat. The preparations were then dried in the air and lastly on a water bath at a gentle heat. When dry, they were powdered and extracted with boiling ether in a fat extractor for several hours, to insure complete freedom from fat. Ultimately, the products for analysis were dried at 105° C. *in vacuo* until of constant weight.

In all, seven preparations of casein were made for analysis, as follows:

No. I. From fresh milk, precipitated twice with hydrochloric acid.
" II. From fresh milk, precipitated twice with acetic acid.
" III. From skimmed milk, precipitated three times with acetic acid.
" IV. A portion of No. III, precipitated a fourth time with acetic acid.
" V. From skimmed milk, precipitated three times with hydrochloric acid.
" VI. A portion of No. V, precipitated a fourth time with hydrochloric acid.
" VII. From skimmed milk, precipitated four times with hydrochloric acid.

The methods of analysis were essentially the same as those employed by Kuhne and Chittenden in the analysis of the various albumose bodies. Carbon and hydrogen were determined by combustion with oxygen in an open tube, the gases passing over a long layer of granular oxide of copper at a bright red heat, a layer of lead chromate at a dull red heat and a roll of freshly reduced metallic copper. Nitrogen was determined as nitrogen gas by combustion with oxide of copper, the gases passing over a long anterior layer of heated oxide, a short layer of metallic copper and a final layer of oxide of copper. The tube was exhausted with a Sprengel pump before and after the combustion and the nitrogen was collected in a Schiffs' azotometer, provided with a jacket tube for rapid cooling of the gas to a constant temperature. In the determination of sulphur and phosphorus, the substance was fused with a mixture of potassium hydroxide and potassium nitrate (10 grams of the former and 1·5 grams of the latter) in a silver crucible, according to the method, designated by Hammarsten * as 1*a*. In order to economize time, a single fusion was made to serve for both a sulphur and phosphorus determination; in other words, a sufficient amount of casein (usually 1·2 grams) was fused with potassium hydroxide and nitrate, the fused mass dissolved in water, the solution made up to a known volume and then one-half, representing one-half of the original substance, was used for the sulphur, the other half for the phosphorus determination. Both the alkali and the nitrate were free from sulphur and phosphorus; at least to such an extent that in a blank experiment, the resultant solutions gave no precipitate whatever, with either barium chloride or with molybdic solution. The oxidations were made at as low a temperature as possible, except towards the end when the temperature was raised, and occasionally a little more nitrate added, to facilitate complete oxidation.

As the percentage of sulphur was quite an important point, we took particular pains to have the final acid fluid entirely free from nitrate and nitrite, as well as from any excess of hydrochloric acid, so as to avoid as much as possible any solvent action on the barium sulphate. For the determination of sulphur, therefore, the alkaline solution of the fused mass was acidified distinctly with hydrochloric acid and the acid solution evaporated to perfect dryness on the water-bath. In this way the objectionable nitrate and nitrite were removed. The residue was then moistened with hydrochloric acid, taken up in water, and the solution allowed to stand until any chloride of silver present, had settled out. The fluid was then filtered and precipitated as usual with barium chloride. For phos-

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* Zeitschrift fur physiologische Chemie, Band ix, p. 289.

ANALYSIS OF CASEIN No. I.

No.	Sub-stance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	$Mg_2P_2O_7$ after fusion with $KOH+KNO_3$. gram.	P %	Ash found. gram.	Ash. %
						c. c.	T. ° C.	Pressure mm.							
I	0·4104	0·2605	7·05	0·7935	52·72	---	----	----	----	------	----	------	----	---	----
II	0·3499	0·2214	7·03	0·6762	52·70	----	----	----	----	------	- --	------	----	----	----
III	0·4850	----	---	----	----	65·6	20·2	765·2	15·72	------	----	------	----	----	----
IV	0·3751	----	----	----	----	50·5	20·0	763·0	15·80	-----	----	------	----	----	----
V	0·6227	----	----	----	----	----	----	----	----	0·0374	0·82	------	----	----	----
VI	0·6227	----	- --	----	---	- --	----	----	----	------	----	0·0189	0·85	----	----
VII	0·4469	----	----	----	----	----	---	----	----	------	----	------	----	0·0043	0·96
VIII	0·4756	----	----	----	----	----	----	----	----	------	----	------	----	0·0046	0·96

Percentage composition of the ash-free substance.

							Average.
C	53·23	53·21	----	----	----	---	*53·22*
H	7·11	7·09	----	----	----	----	*7·10*
N	----	----	15·89	15·97	----	----	*15·93*
S	----	----	----	----	0·83	----	*0·83*
P	----	----	----	---	----	0·86	*0·86*
O	----	----	---	----	----	----	*22·06*
							100·00

ANALYSIS OF CASEIN NO. II.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with HOH + KNO_3. gram.	S %	$Mg_2P_2O_7$ after fusion with KOH + KNO_3. gram.	P %	Ash found. gram.	Ash. %
						c. c.	T. °C.	Pressure mm.							
I	0·3685	0·2300	6·93	0·7137	52·81	----	----	----	----	------	----	------	----	----	----
II	0·4938	0·3120	7·02	0·9590	52·96	----	----	----	----	- ---	----	--- ---	- -	----	----
III	0·3653	----	----	----	----	49·2	21·2	758·2	15·66	------	----	------	--	----	----
IV	0·3542	----	----	----	----	47·5	20·6	756·5	15·77	------	---	------	----	----	----
V	0·5986	----	----	----	----	----	----	----	----	0·0319	0·73	- ----	----	----	----
VI	0·7408	----	----	----	----	----	----	----	----	0·0417	0·77	------	---	----	----
VII	0·5986	----	----	----	----	----	----	----	----	------	----	0·0187	0·87	----	----
VIII	0·7408	----	----	----	----	---	----	----	----	--- --	----	0·0234	0·88	----	----
IX	0·5536	----	----	----	----	----	----	----	----	------	----	------	----	0·0035	0·63
X	0·5324	----	----	----	----	----	----	----	----	------	----	--- --	----	0·0030	0·56

Percentage composition of the ash-free substance.

									Average.
C	53·12	53·27	----	----	----	----	----	----	*53·19*
H	6·97	7·06	-	--	----	----	----	----	*7·01*
N	----	----	15·75	15·86	----	----	----	----	*15·80*
S	----	--	----	----	0·74	0·78	----	----	*0·76*
P	----	----	----	--	---	----	0·88	0·89	*0·88*
O	----	----	----	----	----	----	----	---	*22·36*

ANALYSIS OF CASEIN NO. III.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	$Mg_2P_2O_7$ after fusion with $KOH+KNO_3$. gram.	P %	Ash found. gram.	Ash. %
						c. c.	T. °C.	Pressure mm.							
I	0·3865	0·2451	7·04	0·7458	52·58	----	----	----	----	------	----	------	----	----	----
II	0·3805	0·2375	6·94	0·7835	52·57	----	----	----	----	------	----	------	----	----	----
III	0·3566	----	----	---	----	48·3	21·0	757·4	15·74	------	----	------	----	----	----
IV	0·3686	----	---	----	----	51·1	21.0	757.1	15·83	------	----	------	----	----	----
V	0·5466	---	----	----	----	----	----	----	----	0·0350	0·88	------	---	----	----
VI	0·5466	----	----	---	----	----	----	---	----	------	- -	0·0172	0·88	----	----
VII	0·4835	----	----	----	----	----	----	----	----	------	----	------	----	0·0065	1·34
VIII	0·4290	----	----	----	----	----	----	----	----	------	----	------	----	0·0061	1·42

Percentage composition of the ash-free substance.

							Average.
C	53·31	53·30	----	----	----	----	*53·30*
H	7·14	7·04	----	----	----	----	*7·09*
N	----	----	15·95	16·04	----	----	*15·99*
S	----	----	----	----	0·89	----	*0·89*
P	---	----	----	----	----	0·89	*0·89*
O	----	----	----	----	----	----	*21·84*
							100·00

ANALYSIS OF CASEIN NO. IV.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram	C %	N found.			N %	$BaSO_4$ after fusion with KOH + KNO_3. gram	S %	$Mg_2P_2O_7$ after fusion with KOH + KNO_3. gram.	P %	Ash found. gram.	Ash. %
						c. c.	T. °C.	Pressure mm.							
I	0·3276	0·2080	7·02	0·6371	53·03	----	----	----	----	------	---	------	----	----	----
II	0·4500	0·2807	6·93	0·8720	52·84	----	----	----	----	------	----	------	----	----	----
III	0·3642	---	----	----	----	48·5	20·4	764·6	15·66	------	----	------	----	----	----
IV	0·4003	----	----	----	----	54·1	20·6	764·6	15·87	------	----	------	----	----	----
V	0·6124	----	----	----	----	----	---	----	----	0·0350	0·78	------	----	----	----
VI	0·6006	----	----	----	----	----	----	----	----	0·0368	0·84	------	----	---	----
VII	0·6124	----	----	----	----	----	----	----	----	------	----	0·0189	0·86	----	----
VIII	0·6006	----	----	----	----	----	----	----	----	------	----	0·0181	0·87	----	----
IX	0·3887	----	----	----	----	----	----	---	----	------	----	------	----	0·0034	0·87
X	0·3802	----	----	----	----	-- -	----	----	----	------	----	------	----	0·0029	0·76

Percentage composition of the ash-free substance.

									Average.
C	53·49	53·30	----	--	----	----	----	----	*53·39*
H	7·11	7·02	----	----	----	----	----	----	*7·06*
N	----	----	15·77	15·98	- --	----	----	----	*15·87*
S	----	----	----	----	0·79	0·85	---	----	*0·82*
P	----	----	----	----	----	----	0·87	0·88	*0·87*
O	----	----	----	----	----	----	----	---	*21·99*
									100·00

ANALYSIS OF CASEIN No. V.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	$Mg_2P_2O_7$ after fusion with $KOH+KNO_3$. gram.	P %	Ash found. gram.	Ash. %
						c. c.	T. °C.	Pressure mm.							
I	0·5970	0·3813	7·09	1·1575	52·87	----	----	----	----	------	----	------	----	----	----
II	0·6132	----	----	1·1934	52·91	----	----	----	----	------	----	------	----	----	----
III	0·3084	0·1950	7·02	0·5960	52·70	----	----	----	----	------	----	------	----	----	----
IV	0·3663	----	----	----	----	48·8	18·4	756·9	15·57	------	----	------	----	----	----
V	0·3828	----	----	----	----	51·4	19·4	756·7	15·67	------	----	------	----	----	----
VI	0·8350	----	----	----	----	----	----	----	----	0·0449	0·74	------	----	----	----
VII	0·8350	----	----	----	----	----	----	----	----	------	----	0·0249	0·83	----	----
VIII	0 5279	----	----	----	----	----	----	----	----	------	----	------	----	0·0045	0·85
IX	0·5816	----	----	----	----	----	----	----	----	------	----	------	----	0·0046	0·86

Percentage composition of the ash-free substance.

								Average.
C	53·31	53·35	53·14	----	----	---	----	*53·27*
H	7·15	----	7·08	----	----	----	---	*7·11*
N	----	----	----	15·70	15·80	----	----	*15·75*
S	----	----	----	--	----	0·75	----	*0·75*
P	---	----	----	----	----	----	0·84	*0·84*
O	----	----	----	----	----	----	-- -	*22·28*
								100·00

ANALYSIS OF CASEIN NO. VI.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	$Mg_2P_2O_7$ after fusion with $KOH+KNO_3$. gram.	P %	Ash found. gram.	Ash. %
						c. c.	T. °C.	Pressure mm.							
I	0·3774	0·2357	6·94	0·7302	52·76	----	----	----	----	-- ---	----	------	----	----	--
II	0·3643	0·2294	6·99	0·7040	52·69	----	----	----	----	--- --	----	--- --	---	----	----
III	0·3520	----	----	----	----	47·8	19·6	760·3	15·92	------	----	------	----	----	----
IV	0·3640	----	----	----	---	49·7	20·4	760·3	15·96	------	---	------	----	----	----
V	0·6219	----	----	----	----	----	----	---	----	0·0365	0·80	------	----	----	----
VI	0·6220	----	---	----	----	----	----	----	----	0·0420	0·92	------	- --	----	----
VII	0·6219	----	----	----	----	----	----	---	----	--- --	----	0·0195	0·87	----	----
VIII	0·6220	----	----	----	----	----	----	----	----	--- --	----	0·0193	0·85	----	---
IX	0·4436	----	----	----	----	----	----	----	----	------	----	--- --	----	0·0046	1·03
X	0·4398	----	----	---	----	----	----	-- -	---	------	----	------	---	0·0045	1·02

Percentage composition of the ash-free substance.

									Average.
C	53·29	53·21	----	----	----	----	- --	----	*53·25*
H	7·00	7·05	----	---	----	----	----	----	*7·02*
N	----	----	16·07	16·10	----	----	----	----	*16·08*
S	----	----	----	----	0·81	0·93	----	----	*0·87*
P	----	----	----	----	----	----	0·88	0·86	*0·87*
O	----	----	---	----	----	----	----	----	*21·91*
									100·00

ANALYSIS OF CASEIN NO. VII.

No.	Substance used. gram	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with KOH + KNO_3. gram.	S %	$Mg_2P_2O_7$ after fusion with KOH + KNO_3 gram.	P %	Ash found. gram.	Ash. %
						c. c	T. °C.	Pressure mm.							
I	0·5031	0·3161	6·98	0·9760	52·90	----	----	------	----	------	----	------	---	----	---
II	0·4063	0·2589	7·08	0·7870	52·82	----	----	------	----	------	----	------	---	-- -	----
III	0·4755	------	----	------	----	64·1	20·0	757·3	15·72	------	----	------	---	----	----
IV	0·3980	------	----	------	----	53·6	19·2	760·5	15·82	------	----	-- ---	---	----	- --
V	0·6782	------	----	------	---	----	----	------	----	0·0429	0·86	------	---	----	----
VI	0·6782	------	----	------	----	----	----	------	----	------	----	0·0210	0·86	---	----
VII	0·4059	------	----	------	----	----	----	------	----	------	----	------	---	0·0056	1·37
VIII	0·4118	- ----	----	------	----	----	----	------	----	------	----	------	---	0·0052	1·26

Percentage composition of the ash-free substance.

							Average
C	53·58	53·49	- ---	----	-----	-----	*53·53*
H	7·07	7·14	-----	-----	--- -	-----	*7·10*
N	-----	-----	15·92	16·01	-----	--- -	*15·96*
S	-----	-----	-----	-----	0·88	-----	*0·88*
P	-----	----	-- --	-- -	- ---	0·88	*0·88*
O	--- -	-- --	-----	-- --	-----	-----	*21·65*
							100·00

phorus, the alkaline fluid was acidified with nitric acid, evaporated to dryness, the residue dissolved in a little water acidified with nitric acid, filtered, and the phosphoric acid precipitated in the usual manner with ammonium molybdate. After standing 24 hours at 40° C. this precipitate was filtered off, dissolved in ammonium hydroxide and the phosphoric acid re-precipitated as ammonio magnesium phosphate and ultimately weighed as magnesium pyrophosphate. The accompanying tables show the results of the analyses of the different samples of casein.

Comparing now the average composition of these different preparations of casein, it is to be seen that they all show a very close agreement throughout. Thus the percentage of phosphorus in the seven preparations varies only from 0·84 to 0·89, sulphur from 0·75 to 0·89, nitrogen from 15·75 to 16·08, hydrogen from 7·01 to 7·11 and carbon from 53·19 to 53·53; or leaving out one preparation which for some reason showed a high content of carbon, from 53·19 to 53·39 per cent. The results therefore show a constancy in composition fully as marked as observed by Hammarsten and thus tend to confirm the latter in the view that casein is a single body of definite composition.

Comparing our results collectively, with those obtained by Hammarsten (see table showing average composition), we find a fairly close agreement throughout, although minor differences are to be observed. First, all of our preparations show a content of carbon somewhat higher than found by Hammarsten. The latter investigator found the carbon in his preparations to vary from 52·78 to 53·09 per cent., while in all of our preparations, the content of carbon calculated to the ash-free substance is above 53 per cent. The possibility of our preparations still containing some fat was rendered improbable by the thorough treatment with ether which they had received, and further by the fact that the nitrogen in our preparations was also somewhat higher than found by Hammarsten. One of the preparations, however, with a high content of carbon, was extracted again for several hours with boiling ether, but on analysis the content of carbon was found unchanged. The content of phosphorus agrees exactly with Hammarsten's results, while the sulphur is, on an average, 0·1 per cent. higher. There is nothing in the content of sulphur, therefore, to even suggest confirmation of Danilewsky's views. The amount of ash in our preparations was somewhat larger than found by Hammarsten and further, there is no especial connection to be seen between the content of ash and the precipitant used; the

TABLE SHOWING THE AVERAGE COMPOSITION OF THE CASEINS.

	No. I. twice precipitated with HCl.	No. II. twice precipitated with CH_3-COOH.	No. III. 3 times precipitated with CH_3-COOH.	No. IV. 4 times pre- with cipitated CH_3-COOH.	No V. 3 times precipitated with HCl.	No. VI. 4 times precipitated with HCl.	No. VII. 4 times precipitated with HCl.	Average of Nos I–VII	Average of Hammarsten's results *	According to Ritthausen †
C	53·22	53·19	53·30	53·39	53·27	53·25	53·53	53·30	52·96	54·22
H	7·10	7·01	7·09	7·06	7·11	7·02	7·10	7·07	7·05	7·17
N	15·93	15·80	15·99	15·87	15·75	16·08	15·96	15·91	15·65	15·49
S	0·83	0·76	0·89	0·82	0·75	0·87	0·88	0·82	0·72	0·91
P	0·86	0·88	0·89	0·87	0·84	0·87	0·88	0·87	0·85	----
O	22·06	22·36	21·84	21·99	22·28	21·91	21·65	22·03	22·78	----
ash	0·96	0·59	1·33	0·81	0·85	1·02	1·31	0·98		

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* See Zeitschrift fur physiologische chemie, Band vii, p. 269.

† Calculated by Ritthausen from analysis of the copper compound of casein, average of three results. See Jahresbericht fur Thierchemie, 1873, p. 28.

amount in the acetic acid precipitate being fully as large as in the casein precipitated by hydrochloric acid.

Compared with Ritthausen's results (see table showing average composition), obtained by analysis of the copper compound of casein, the percentage of carbon comes very much too high. It is questionable, however, how close a comparison should be drawn between indirect results obtained by analysis of a metallic compound of casein and those obtained by analysis of casein itself.

In conclusion then, we must affirm that our results accord closely with those of Hammarsten's, while the two together make it very improbable that in casein we have to do with a substance composed of two bodies of unlike composition.

Digestion of Casein and Formation of Caseoses.

In the digestion of casein with pepsin-hydrochloric acid, the casein was prepared by precipitation and reprecipitation with acetic acid, and rendered as pure as possible by thorough washing with water. While still moist it was placed in 0·4 per cent. hydrochloric acid, as preliminary to its treatment with pepsin. Pure pepsin solution, free from peptone and albumose, was prepared from the mucous membrane of pig's stomachs by the method already described.*

Digestion A.

1300 grams of moist casein in 4 litres of 0·4 per cent. hydrochloric acid were brought to a temperature of 45° C. and 600 c.c. of pure pepsin solution added. The mixture was kept at a temperature of 45° C. throughout the digestion. The casein began almost immediately to swell up and in less than an hour the entire mixture was converted into a semi-solid, jelly-like mass. Thereupon, one litre more of 0·4 per cent. hydrochloric acid was added, together with a little more pepsin solution. At the end of three hours, the mixture was quite fluid, but contained considerable gelatinous matter in suspension. Neutralization of a filtered portion, produced no precipitate whatever. The addition of crystals of sodium chloride gave a heavy white precipitate and the filtrate from this precipitate gave a further precipitate on the addition of acetic acid. At the end of four hours, the entire mixture was made neutral with sodium hydroxide and then filtered through paper. The undigested residue, when dry, amounted to about 30 or 40 grams. This residue of so-called casein dyspeptone, which appeared in every digestion in greater or less quantity, was apparently wholly insoluble in fresh portions of gastric juice and was similar in its reactions to the like-body previously described by Lubavin.[*] When fresh, it appeared as a more or less jelly-like mass, much like starch paste. It was readily soluble in dilute alkalies and precipitated by neutralization, but insoluble in excess of acid. It was precipitated from its solution in dilute sodium hydroxide by addition of salt in substance. This body we did not attempt to study further, but hope to do so later.

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* See the preceding article on egg-albumin and albumoses.

The filtrate from the undigested residue, when cold, was not perfectly clear but became so on the application of a gentle heat. On boiling the solution, a very slight precipitate was formed. The addition of acids, hydrochloric, nitric or acetic, either concentrated or dilute, caused a heavy white precipitate, not wholly soluble in excess of acid, even of concentrated hydrochloric. The precipitate was likewise more or less permanent when warmed or even boiled with the acid. In the filtrate from the precipitate produced by acetic acid, the addition of potassium ferrocyanide gave no precipitate. The precipitate produced by acids was readily soluble in dilute alkalies. On addition of nitric acid, of any strength, and the application of heat even to boiling, the mixture turned first rose color then reddish, and as the boiling was continued the color deepened and finally became brownish red. The change from rose color to brown also takes place in the cold. With concentrated nitric acid the color is nearer the yellow of the xanthoprotein reaction, but still shows plainly the brown or reddish tinge.

For separation of the individual caseoses, the entire solution without being concentrated, was saturated with sodium chloride, by which an exceedingly heavy precipitate, more or less curdy, was obtained, which was finally filtered off and washed with saturated salt solution. The washing ,was made more thorough by grinding the mass with the salt solution in a mortar. This precipitate, by analogy, would naturally be composed mainly of a body corresponding to protoalbumose with possibly something corresponding to heteroalbumose. The precipitate was washed thoroughly with saturated salt solution, dissolved in water, filtered, and again precipitated by saturation of the fluid with sodium chloride. All of the substance, however, was not reprecipitated; quite a little remained in the salt-saturated fluid and was thrown down as a white curdy precipitate by the addition of a little acetic acid, evidently some protocaseose not precipitated by salt alone. The main precipitate of protocaseose, etc., twice precipitated by salt, was treated with 3 litres of 10 per cent. salt solution, the residue with 3 litres of 5 per cent. salt solution and the residue still remaining, with 3 litres of water. In this manner, all of the proto and heterocaseose was dissolved, leaving a small residue wholly insoluble in dilute salt solutions and in water; presumably dyscaseose. The latter, however, was in exceedingly small quantity. It was dissolved in 0·2 per cent. hydrochloric acid and reprecipitated by neutralization of the solution with sodium carbonate.

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[*] Ueber die Künstliche Pepsin-Verdauung des caseins, etc, Hoppe-Seyler's Med. Chemische Untersuchungen, p 467.

A. *Protocaseose.*

The 5 and 10 per cent. salt solutions of protocaseose together with the aqueous solution, were united and the mixture saturated with sodium chloride. Here, as before, all of the protocaseose was not precipitated; a portion remained in the filtrate and was precipitated only on the addition of a little acetic acid. The main portion of the protocaseose precipitated for the third time with salt in substance, was dissolved in water, the solution filtered and divided into two parts. One part was thymolized and dialyzed in running water until all chlorine was removed from the solution (Protocaseose A 1). The other part was again saturated with salt, the precipitate washed with saturated salt solution, then dissolved in water and like the former dialyzed until all chlorine was removed from the solution (Protocaseose A 2). In protocaseose 2, there was more evidence of the presence of a body resembling heteroalbumose than in No. 1. Thus in No. 2, quite a little gummy substance separated from the solution on dialysis, but the amount even here was not large. When the dialysis was finished, both solutions were perfectly neutral to test papers and in both cases the protocaseose was separated from the clear fluid by evaporation and precipitation with alcohol. For analysis, both products were washed thoroughly with alcohol and ether and finally dried at 105° C. *in vacuo.*

Their composition is shown in the accompanying tables.

As already stated, every time protocaseose was dissolved in water and reprecipitated by saturating the solution with sodium chloride, a certain amount of the substance remained in solution, precipitable only on addition of a little acetic acid. Protocaseose precipitated from the salt-saturated solution in this manner by acetic acid, ANALYSIS OF PROTOCASEOSE A 1.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c c.	T. °C.	Pressure mm.					
I	0·4722	0·2851	6·70	0·8519	49·20	----	----	----	----	------	----	------	----
II	0·3360	-----	---	0·6080	49·34	- --	----	----	----	------	----	-----	----
III	0·3500	-----	----	------	----	43·7	20·4	766·2	14·71	------	----	------	----
IV	0·3850	-----	---	------	----	48·3	19·7	766·2	14·81	-----	----	------	----
V	0·6060	-----	----	------	----	----	----	----	----	0·0380	0·86	------	----
VI	0·5025	-----	---	------	----	----	----	----	----	------	--	0·0312	6·20

Percentage composition of the ash-free substance.

						Average.
C	52·44	52·57	----	----	----	*52·50*
H	7·15	----	----	----	----	*7·15*
N	----	----	15·68	15·78	----	*15·73*
S	----	----	----	----	0·96	*0·96*
O	----	----	----	----	----	*23·66*
						100·00

ANALYSIS OF PROTOCASEOSE A 2.

Substance used. gram	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found gram.	Ash. %	$BaSO_2$ from the ash gram.	S of Ash % of Substance.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of Ash. %
					c. c.	T. °C.	Pressure mm.								
0·3729	0·2284	6·80	0·6895	50·42	----	----	----	----	----	----	------	------	------	----	-----
0·3934	0·2366	6·69	0·7265	50·38	----	----	------	----	----	----	------	------	------	----	-----
0·3636	------	----	------	----	45·8	20·1	766·1	14·85	----	----	------	------	------	----	-----
0·3562	------	----	------	----	44·9	20·2	764·2	14·82	----	----	------	------	------	----	-----
0·4923	------	----	-----	----	----	----	------	----	0·0316	6·42	-----	------	------	----	-----
0·4923	-----	----	------	----	----	----	------	----	----	----	0·0231	0·64	------	----	-----
0·5925	------	----	------	----	----	----	------	----	----	--	-----	------	0·0659	1·52	0·88

Percentage composition of the ash-free substance.

						Average.
C	53·87	53·83	----	----	----	*53·85*
H	7·27	7·16	----	---	----	*7·21*
N	----	----	15·86	15·83	----	*15·84*
S	----	----	---	----	0·98	*0·98*
O	----	----	----	----	----	*22·12*
						100·00

was found to be quite different in its nature from protoalbumose or protoglobulose. The two latter undoubtedly combine with acetic acid, when precipitated from a salt-saturated fluid; the compound, however, is readily and completely soluble in water. With protocaseose on the other hand, acetic acid produces a precipitate, not only insoluble in the salt-saturated fluid, but also more or less insoluble in water containing a little acid. It is easily soluble in dilute alkali and alkali carbonate, and is not precipitated by neutralization with hydrochloric or acetic acid. Addition of acid, however, beyond neutralization immediately causes precipitation of the caseose.

Protocaseose once precipitated with salt and which on the second precipitation, failed to separate from the salt-saturated fluid, was precipitated by a little acetic acid, washed somewhat with water, dissolved in very dilute sodium carbonate solution, neutralized and then dialyzed in running water for nearly a week. The solution was then perfectly neutral to test papers and was likewise perfectly clear. It was evaporated to a syrup and the caseose precipitated by alcohol. After being washed with alcohol and ether, it was dried at 105° C. *in vacuo* until of constant weight, and then analyzed with the following results:

Protocaseose A 3.

I. 0·3703 gram substance gave 0·2160 gram $H_2O = 6·48$ per cent. H and 0·6500 gram $CO_2 = 47·86$ per cent. C.

II. 0·4200 gram gave 50·9 c. c. N at 21·2° C. and $765·0^{mm}$ pressure = 14·21 per cent. N.

III. 0·7053 gram fused with KOH and KNO_3 gave 0·0418 gram $BaSO_4$ = 0·81 per cent. S.

IV. 0·4076 gram gave 0·0386 gram ash = 9·47 per cent.

The ash-free substance therefore contained

52·59% C. 7·17% H. 15·70% N. 0·90% S.

Like all of the caseose bodies, this contained a large percentage of ash in spite of its long continued dialysis. The ash was mainly calcium phosphate with some oxide of iron, obtained in part doubtless from the salt used in precipitation. It is not difficult to see how protocaseose, precipitated by salt alone, should take up and retain semi-mechanically considerable inorganic matter. But in the present case, where the great mass of the albuminous substance has been precipitated by the salt added, it seems somewhat surprising that the caseose should separate from the clear fluid in the presence of considerable acetic acid with such a large percentage of adherent mineral matter, unless the latter is chemically combined with the albuminous substance. Oft-repeated and long continued dialysis appears to have but little influence in diminishing the amount of this impurity. Our experience has taught us that where the caseoses have once been brought in contact with lime salts, reprecipitation and other methods of purification avail but little.

But very little heterocaseose was found in this digestion, not as much as was found in some of the others, later on. Still, during the first dialysis of the protocaseose, some little heterocaseose separated from the fluid, as the last traces of salt dialyzed out.

A. *Deuterocaseose.*

Deuterocaseose was separated from the filtrate from the first sodium chloride precipitate of protocaseose, by acetic acid. As a study of the reactions of protocaseose had shown plainly that this body is never completely precipitated by salt alone, a little acetic acid was added to the salt-saturated filtrate, and the precipitate, presumably a mixture of proto and deuterocaseose, thrown away. The remaining deuterocaseose was then precipitated by adding about 200 c.c. of a salt-saturated acetic acid (30 per cent. acetic acid) to the fluid. The total volume of the mixture was nearly 16 litres. In this manner an abundance of a finely divided precipitate was obtained, which at first, seemed insoluble in water and in dilute sodium chloride. It was readily soluble in water containing a trace of alkali and was not precipitated by neutralization. On being washed, however, with a saturated salt solution for some time, the washings were found to have dissolved considerable of the substance, which could be precipitated from the solution by strong acetic acid. Further, after being washed with salt solution, the substance remaining appeared quite noticeably soluble in water. Evidently then, this body on being washed more or less free from acid, becomes soluble in water to a certain extent, its aqueous solution then giving a strong reaction with acetic acid and potassium ferrocyanide. The great bulk of the precipitate was therefore washed with saturated salt solution until the washings were nearly free from acid; then, having become partially soluble in water, it was placed in about 2 litres of water, the solution ultimately saturated with sodium chloride and the caseose again precipitated by addition of about 20 c. c. of acetic acid. This second precipitate was washed somewhat with salt solution and finally with a large volume of water. At first, the washings gave no reaction with

ANALYSIS OF DEUTEROCASEOSE A.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm	N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
I	0·4027	0·2261	6·23	0·6850	46·38	----	----	- ---	----	-----	----	-----	-----
II	0·4002	0·2265	6·27	0·6787	46·24	----	---	- -	----	-----	----	-----	-----
III	0·3736	-----	----	-----	----	45·8	18·0	765·9	14·07	-----	----	- - -	-----
IV	0·3550	-----	----	-----	---	42·4	18·4	766·2	14·17	--- -	----	--- -	-- --
V	0·6024	-----	---	-----	----	---	--	-----	--	0·0294	0·67	-- -	-----
VI	0·6030	-----	----	----	----	-- -	- -	---	----	0·0299	0·68	-----	-----
VII	0·3489	-----	----	-----	---	---	----	--- -	-	- ---	---	0·0358	10·26

Percentage composition of the ash-free substance.

							Average
C	51·66	51·52	----	----	----	----	*51·59*
H	6·96	7·00	----	----	---	----	*6·98*
N	----	----	15·68	15·78	----	----	*15·73*
S	----	---	----	----	0·74	0·75	*0·75*
O	--	---	----	----	----	----	*25·03*
							100·00

acetic acid and potassium ferrocyanide, but later on, these reagents gave a heavy precipitate, showing plainly that the substance was dissolving. The entire precipitate was thereupon dissolved in very dilute sodium carbonate, the solution made exactly neutral with hydrochloric acid and then dialyzed. After remaining in the dialyzers for nearly a week the fluid was removed, filtered from some heterocaseose which had separated, evaporated to a syrup on the water-bath and precipitated with alcohol. This precipitate was re-dissolved in water, the solution made exactly neutral to test papers and again dialyzed. From this solution, the caseose was finally precipitated by alcohol, after suitable concentration of the fluid, washed with alcohol and ether and dried at 105° C. *in vacuo.* The final solution, prior to precipitation by alcohol, was perfectly neutral and quite clear, showing no evidence of the presence of any heterocaseose.

The composition of the substance is shown in the accompanying table.

Digestion B.

In this digestion, 750 grams of freshly prepared casein were mixed with 4 litres of 0·4 per cent. hydrochloric acid, the mixture warmed at 45° C., and then 800 c. c. of pure pepsin solution added. The mixture was warmed at the above temperature for one hour and a half, then neutralized and filtered, and the clear filtrate saturated with sodium chloride. This precipitate, as in the preceding digestion, was washed thoroughly with saturated salt solution, then successively extracted with 10 and 5 per cent. salt solution and finally with water, leaving a small residue of dyscaseose soluble only in 0·2 per cent. hydrochloric acid. The united filtrates, containing proto and heterocaseose, were again precipitated with salt and then treated as described under protocaseose B.

B. Protocaseose.

The protocaseose formed in this digestion and twice precipitated by salt, was dissolved again in water, filtered through paper and then dialyzed until no chlorine reaction could be obtained with silver nitrate. Quite a little heterocaseose separated from the solution during dialysis, which was removed by filtration. The fluid was then concentrated, the substance precipitated by alcohol, again dissolved in water and dialyzed. This time, as no heterocaseose separated from the fluid, the solution was concentrated, precipitated

ANALYSIS OF PROTOCASEOSE B 1.

Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %	$BaSO_4$ from the Ash. gram.	S of Ash % of Substance.	$BaSO_4$ after fusion with KOH + KNO_3. gram.	S %	S after deducting S of Ash. %
					c. c.	T. °C.	Pressure mm.								
0·3844	0·2268	6·56	0·6892	48·89	----	----	------	----	----	----	------	--- --	------	----	-----
0·4268	0·2504	6·51	0·7642	48·82	----	----	------	----	----	----	------	------	--- --	----	-----
0·4758	------	----	------	---	58·5	19·6	760·8	14·42	----	----	------	- -	------	----	-----
0·4054	------	----	------	----	50·3	20·2	759·8	14·50	----	----	------	------	---- -	----	----
0·3629	------	----	------	---	----	----	------	----	0·0278	7·66	------	------	------	-- -	-----
0·3629	------	----	------	----	----	--	------	----	----	----	0·0200	0·75	------	----	- -
0·6254	------	--	--- --	----	----	---	------	----	----	----	------	------	0·0696	1·52	0·77

Percentage composition of the ash-free substance.

						Average.
C	52·94	52·88	----	----	----	*52·91*
H	7·09	7·04	----	----	----	*7·06*
N	----	----	15·61	15·69	----	*15·65*
S	----	----	----	----	0·90	*0·90*
O	----	----	----	----	- --	*23·48*
						100·00

ANALYSIS OF PROTOCASEOSE B 2.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c	T. °C.	Pressure mm.					
I	0·5084	0·2897	6·33	0·8808	47·44	----	----	------	----	------	----	------	----
II	0·3520	0·2001	6·31	0·6130	47·49	---	----	------	----	--- --	----	------	----
III	0·4521	------	----	------	----	56·0	19·6	763·5	14·58	------	----	------	----
IV	0·5010	------	----	------	-- -	62·5	20·4	762·3	14·62	-- ---	----	------	----
V	0·6021	------	----	------	----	----	----	--- --	----	0·0367	0·83	------	----
VI	0·6010	------	----	------	----	----	---	- ----	---	0·0353	0·81	--- --	----
VII	0·3940	------	----	------	----	----	----	-----	----	------	----	0·0389	9·87

Percentage composition of the ash-free substance.

							Average.
C	52·41	52·46	----	----	----	----	*52·43*
H	7·02	7·00	----	----	----	----	*7·01*
N	----	----	16·17	16·21	----	----	*16·19*
S	----	----	----	----	0·91	0·89	*0·90*
O	----	----	----	----	----	----	*23·47*
							100·00

with alcohol and the substance finally dried at 105° C. *in vacuo* (Protocaseose B 1). Its composition is shown in the accompanying table.

Such portion of the protocaseose as was not precipitated the second time by salt alone, was precipitated by a little acetic acid. At first, the precipitated substance was insoluble in water (no reaction with acetic acid and potassium ferrocyanide), but after being washed with salt solution until the acid reaction had nearly disappeared, it then dissolved quite appreciably in water, as evidenced by the reaction with acetic acid and potassium ferrocyanide. The bulk of the precipitate, after being washed, was dissolved in a little very dilute sodium carbonate, the solution made exactly neutral with hydrochloric acid and then dialyzed until all chlorine was removed from the solution. The caseose, after concentration of the solution, was precipitated by alcohol, washed with alcohol and ether and finally dried at 105° C. *in vacuo*. The composition of the substance (Protocaseose B 2) is shown in the accompanying table.

B. Deuterocaseose.

Deuterocaseose was separated from the filtrate from the first sodium chloride precipitate, in the same manner as in the preceding digestion. Like A. deuterocaseose, this precipitate was at first insoluble in salt solution and in water, but after being washed for some time with saturated salt solution, it was found to be gradually dissolved, as shown by the rapid disappearance of the precipitate and the pronounced reaction with acetic acid and potassium ferrocyanide in the wash-fluid. Evidently the acid is easily removed from the compound by simple washing and when that has been effected, the substance becomes soluble, or else the acid compound is more insoluble in water containing a little free acid, than in water or salt solution alone. Hydrochloric acid and acetic acid seem to act alike. The substance is readily soluble in dilute sodium carbonate and is not precipitated by neutralization, but is quickly thrown down by a slight excess of hydrochloric or acetic acid. This compound was not analyzed, but was used in studying the reactions to be described later.

Digestion C.

In both of the preceding digestions, the products formed resulted from the action of an exceedingly vigorous pepsin mixture. In the present case, the pepsin solution employed was much weaker and the pepsin-casein mixture was warmed at 45° C. for several days instead of hours.

750 grams of casein were employed and 4 litres of 0·4 per cent. hydrochloric acid, to which was added a reasonable amount of pure pepsin solution. About an hour after the addition of the latter the mass began to gelatinize, and at the end of 18 hours the whole mixture was a perfectly stiff jelly. Thereupon, 2 litres more of 0·4 per cent. acid were added together with a little pepsin. The mixture was then kept at 40–45° C. for three days longer. Quite a large residue of undigested matter remained, semi-gelatinous and soluble only in alkalies The mixture was then neutralized and filtered. The filtrate contained considerable caseose, as evidenced by the heavy precipitate obtained on saturating a portion with sodium chloride. In the preceding digestions, the caseoses were separated directly from the dilute solutions, without previous concentration, thereby avoiding any possible change due to the action of heat. In the present case, however, the perfectly neutral solution was evaporated to a small volume and then all of the caseoses were directly precipitated by saturating the fluid with ammonium sulphate. The precipitate produced was exceedingly gummy, but was washed as thoroughly as possible by trituration with a saturated solution of ammonium sulphate. The caseoses were then dissolved in water, the solution filtered from the small residue of insoluble matter and saturated with sodium chloride. The protocaseose so separated was freed from heterocaseose, etc. by repeated precipitation and dialysis.

Carbon and hydrogen were determined in a portion of the dried substance (Protocaseose C 1) with the following results:

I. 0·5107 gram substance gave 0·3101 gram H_2O=6·74 per cent. H and 0·9386 gram CO_2=50·11 per cent C.

II. 0·4038 gram gave 0·0194 gram ash = 4·80 per cent.

The ash-free substance would, therefore, contain 52·64 per cent. of carbon and 7·08 per cent. of hydrogen.

In the filtrate from the first salt precipitate, the protocaseose remaining was precipitated by the addition of a little salt-saturated acetic acid. The precipitate after being washed was dissolved in a little dilute sodium carbonate, the solution neutralized and dialyzed.

As no heterocaseose separated from the solution, it was concentrated and then precipitated by alcohol. After purification by resolution, dialysis, etc., it was dried at 105° C. *in vacuo* and analyzed with the results shown in the accompanying table (Protocaseose C 2).

ANALYSIS OF PROTOCASEOSE C 2.

No.	Substance used gram.	H_2O found gram.	H %	CO_2 found. gram	C %	N found.			N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. °C.	Pressure mm.					
I	0·4080	0·2658	6·51	0·7028	46·90	----	----	----	----	------	----	------	----
II	0·4235	0·2458	6·45	0·7258	46·74	----	---	----	----	------	----	------	----
III	0·3395	------	----	------	----	41·4	19·2	759·4	14·30	- ----	----	------	----
IV	0·3580	------	----	------	----	43·9	20·1	766·2	14·46	------	----	------	----
V	0·6008	------	---	------	----	----	----	----	---	0·0315	0·72	------	----
VI	0·4068	------	----	------	----	----	----	----	----	------	----	0 0386	9·48
VII	0·4314	------	----	------	---	----	----	----	----	-- ---	---	0·0399	9·24

Percentage composition of the ash-free substance.

						Average.
C	51·81	51.65	----	----	----	*51·73*
H	7·18	7·12	----	----	- --	*7·15*
N	----	---	15·77	15·93	----	*15·85*
S	---	----	----	---	0·79	*0·79*
O	----	----	----	----	----	*24·48*
						100·00

Deuterocaseose was then separated from the filtrate from the acetic acid precipitate, by saturation of the fluid with ammonium sulphate, as recommended by Neumeister* for deuteroalbumose. It was then purified by dialysis, etc., and its reactions carefully studied. So far as we could see, it differed from deuterocaseose A and B in two respects only, but these points showed so marked a difference it was plainly evident that the deuterocaseose separated by ammonium sulphate was quite different from deuterocaseose A, separated by acetic acid. Thus deutero C was not precipitated at all in an aqueous solution by acetic acid, nor by acetic acid and potassium ferrocyanide, neither was its aqueous solution precipitated by cupric sulphate. The significance of these points of difference will be discussed later on. After studying the reactions, there was not enough substance remaining for analysis.

Digestion D.

In this digestion, 2 kilos of freshly prepared casein were used, together with 6 litres of 0·4 per cent. hydrochloric acid and an appropriate quantity of strong pepsin solution. The mixture was warmed at 45° C. for five hours, then neutralized and filtered from the semi-gelatinous residue.

D. *Protocaseose.*

The neutral fluid was concentrated to about 1½ litres and then filtered from the slight flocculent precipitate which had formed. Saturation of the fluid with sodium chloride gave an exceedingly heavy precipitate, somewhat more gummy than usual. The entire fluid, however, was only partially saturated with salt, with a view to see whether the precipitate produced in this manner would agree wholly with the precipitate produced on complete saturation. Thus a fractional precipitation was made, in which the first fraction represents that portion of the caseose precipitated by about two-thirds saturation of the fluid with salt. This precipitate was therefore filtered off, washed as usual, dissolved in water and re-precipitated with salt. As dyscaseose was generally found only in traces, the precipitate, after being washed, was dissolved at once in water and dialyzed until all chlorine was removed from the solution. On opening the dialyzing tubes, quite a large quantity of heterocaseose was found adherent to the sides of the paper. The clear solution of

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* See Zeitschrift fur Biologie, Band xxiii, p. 381.

ANALYSIS OF PROTOCASEOSE D 1.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH+KNO_3$. gram.	S %	Ash found gram.	Ash %	$Mg_2P_2O_7$ from the ash. gram.	P of ash, % of substance.	$Mg_2P_2O_7$ after fusion with $KOH+KNO_3$ gram.	P %	P after deducting P of ash, %
						c. c.	T. °C.	Pressure. mm.										
I	0·5211	0·3268	6·95	0·9890	51·80	--	--	----	---	-----	--	----	--	---	----	-----	--	----
II	0·4062	0·2508	6·86	0·7745	51·99	--	--	----	----	-- --	--	----	--	- -	- --	-----	--	----
III	0·4528	----	--	----	---	59·1	18·4	762·3	15·40	-----	--	----	--	-- -	----	-----	--	----
IV	0·5675	----	--	----	---	48·8	18·5	762·3	15·50	-----	--	----	--	----	----	-----	--	----
V	0·6014	----	--	----	----	--	--	----	----	0·0348	0·79	- --	--	----	----	-----	--	----
VI	0·6004	----	--	----	----	--	--	----	----	0·0374	0·85	----	--	----	---	-----	--	----
VII	0·4473	----	--	----	----	--	--	----	---	-----	--	0·0162	3·84	----	----	-----	--	----
VIII	0·4521	----	--	----	----	--	--	---	----	-- --	--	0·0168	3·71	----	----	-----	--	----
IX	0·4049	----	--	---	----	--	--	----	----	-----	--	----	--	0·0099	0·68	-----	--	----
X	0·6014	----	--	----	----	--	--	----	----	--- -	--	----	--	----	----	0·0149	0·69	0·01
XI	0·6004	----	--	----	----	--	--	----	----	-----	--	----	--	----	----	0·0147	0·68	0

Percentage composition of the ash-free substance.

							Average.
C	53·84	54·03	----	----	----	- -	*53·93*
H	7·21	7·12	----	----	----	----	*7·17*
N	----	----	16·00	16·10	----	----	*16·05*
S	----	----	----	-- -	0·82	0·88	*0·85*
P	----	----	----	----	----	----	*0*
O	----	----	----	----	----	----	*22·00*
							100·00

ANALYSIS OF PROTOCASEOSE D 2.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found gram.	Ash %
						c. c	T. °C.	Pressure mm.					
I	0·4196	0·2511	6·65	0·7607	49.48	- --	---	-----	-----	-----	----	--- -	-- -
II	0·3511	0·2103	6·65	0·6378	49.53	----	---	-----	----	-----	----	-----	----
III	0·4789	------	----	--- --	----	60·5	18·8	762·1	14·88	-----	-	--- -	----
IV	0·3626	-- ---	----	--- --	----	45·7	19·2	762·1	14·83	-----	----	- - -	----
V	0·6129	------	----	------	----	----	---	-----	-----	0·0414	0·92	-----	----
VI	0·6350	-- --	----	------	----	----	---	-----	-----	0·0482	1·04	-----	----
VII	0·4220	------	----	------	----	----	---	----	-----	- ---	----	0·0267	6·32
VIII	0·4186	-- ---	----	------	----	----	---	-----	-----	-----	- -	0·0270	6·45

Percentage composition of the ash-free substance.

							Average
C	52·79	52·88	----	----	----	----	*52·84*
H	7·10	7·10	----	----	----	---	*7·10*
N	----	----	15·89	15·84	----	----	*15·86*
S	----	----	----	---	0·99	1.10	*1·04*
O	- -	- --	----	----		----	*23·16*
							100·00

protocaseose was concentrated, precipitated with alcohol, the precipitate dissolved in water, again precipitated with salt in substance, the precipitate dialyzed, this time without showing any heterocaseose, and finally precipitated with alcohol, washed with alcohol and ether and dried at 105° C. *in vacuo* (Protocaseose D 1). The product was analyzed with the results shown in the preceding table.

On adding more sodium chloride to the first filtrate from the above precipitate, thereby completely saturating the solution, a second precipitate of protocaseose was obtained, which was purified in the same manner as the preceding preparation. The only difference noticed while purifying the substance was that, on dialysis, nothing corresponding to heterocaseose separated from the fluid. After final washing with alcohol and ether, the substance was analyzed with the results shown in the accompanying table (Protocaseose D 2).

The difference in the results will be discussed later on.

The original salt-saturated filtrate was precipitated with a little acetic acid, and the protocaseose so precipitated dissolved in dilute sodium carbonate. The solution was then neutralized and dialyzed in running water until all chlorine was removed. The substance was then separated by precipitation with alcohol, and ultimately purified as described previously (Protocaseose D 3). The addition of a little more acetic acid to the acetic acid and salt-saturated filtrate from the above, gave a still further precipitate of caseose; presumably protocaseose with perhaps a trace of deuterocaseose, which was filtered off, washed with saturated salt solution and then freed from acid and purified in the same manner as the preceding preparation (Protocaseose D 4).

The two last products, after being dried at 105° C. *in vacuo*, were analyzed with the results shown in the following tables.

Comparing these two tables, it is seen that the difference in percentage composition of the two ash-free substances is not very great, and taking into consideration the large percentage of ash, it is probable that the two latter precipitates have approximately the same composition.

D. *Deuterocaseose.*

The filtrate, from which certainly all protocaseose had been removed by acetic acid, and in fact nearly everything precipitable by acid from the salt-saturated fluid, was treated with ammonium sulphate in substance. A gummy precipitate resulted, which naturally enclosed considerable salt, and which for purification was dis-

Analysis of Protocaseose D 3.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram	Ash %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram	S %
						c. c.	T. °C.	Pressure m m.					
I	0·4072	0·2416	6·59	0·7199	48·21	----	----	----	----	----	----	------	----
II	0·4384	0·2622	6·64	0·7767	48·31	----	----	----	----	----	----	-- ---	----
III	0·3562	----	----	----	---	44·7	18·4	766·6	14·89	----	----	------	----
IV	0·3556	----	----	----	----	45·4	18·1	765·8	15·01	----	----	------	----
V	0·4036	----	----	----	----	----	----	---	----	0·0296	7·33	-- --	----
VI	0·4614	----	----	----	----	----	----	----	----	----	----	0·0337	1·00
VII	0·3984	----	----	----	----	----	----	----	----	----	----	0·0279	0·96

Percentage composition of the ash-free substance.

							Average.
C	52·01	52·10	----	----	----	----	*52·05*
H	7·11	7·16	---	----	----	-- -	*7·13*
N	----	----	16·07	16·18	----	----	*16·13*
S	----	----	----	----	1·08	1·04	*1·06*
O	-- -	----	----	----	----	----	*23·64*
							100·00

ANALYSIS OF PROTOCASEOSE D 4.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with $KOH + KNO_3$. gram.	S %	Ash found. gram.	Ash %
						c. c.	T. ° C.	Pressure mm.					
I	0·3156	0·1907	6·71	0·5816	50·25	----	---	-----	----	------	----	------	----
II	0·4575	0·2763	6·71	0·8408	50·11	----	----	-----	----	------	- -	------	----
III	0·3818	-----	----	-----	----	49·6	18·6	763·1	15·34	---- -	----	------	----
IV	0·3627	-----	----	-----	----	46·8	18·6	766 8	15·30	------	----	------	----
V	0·6093	-----	----	-----	----	----	----	-----	----	0·0418	0·94	-----	----
VI	0·6047	-----	- --	-----	----	----	----	-----	----	0·0412	0·93	--- --	----
VII	0·4237	-----	----	-----	----	----	----	-----	----	------	---	0·0217	5·12
VIII	0·4103	-----	----	-----	----	----	---	- ---	-- -	------	- -	0·0210	5·11

Percentage composition of the ash-free substance.

							Average.
C	52·95	52·81	----	----	-- -	----	*52·88*
H	7·07	7·07	----	----	----	----	*7·07*
N	----	----	16·16	16·11	---	----	*16·13*
S	----	----	----	-	0·99	0·98	*0·98*
O	----	----	----	----	----	----	*22·94*
							100·00

ANALYSIS OF DEUTEROCASEOSE D.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	$BaSO_4$ after fusion with KOH + KNO_3. gram.	S %	Ash found. gram.	Ash %	$Mg_2P_2O_7$ from the ash. gram.	P of ash, % of substance.	$Mg_2P_2O_7$ after fusion with KOH + KNO_3 gram.	P %	P after deducting P of ash %
						c. c.	T. °C.	Pressure. mm.										
I	0·4992	0·3030	6·74	0·9047	49·42	--	--	----	---	-----	--	---	--	----	- --	-----	--	-- -
II	0·3644	0·2200	6·71	0·6591	49·32	--	--	----	----	-----	--	- --	--	--	----	-----	--	----
III	0·4112	----	--	----	----	52·8	18·5	766·1	15·28	-----	--	----	--	----	----	-----	--	----
IV	0·3860	----	--	----	----	49·7	18·0	766·9	15·30	-----	--	----	--	----	---	----	--	---
V	0·6084	----	--	---	----	--	--	----	----	0·0486	1·10	---	--	----	----	-----	--	----
VI	0·6020	----	--	----	---	--	--	----	----	0·0500	1·14	----	--	----	----	-----	--	----
VII	0·4083	----	--	---	---	--	--	----	----	-----	--	0·0191	4·74	----	- --	-----	--	----
VIII	0·4022	----	--	---	---	--	--	----	----	-----	--	0·0189	4·62	----	----	-----		---
VIII*b*	0·4022	----	--	----	----	--	--	----	----	----	-	----	--	0·0097	0·67	-----	--	----
IX	0·6034	----	--	----	----	--	--	----	----	-----	--	----	--	----	----	0·0150	0·69	0·02
X	0·6020	----	--	----	---	--	--	----	----	-----	--	----	--	----	---	0·0151	0·70	0·03

Percentage composition of the ash-free substance.

							Average.
C	51·84	51·74	----	----	----	----	*51·79*
H	7·07	7·04	----	----	----	----	*7·05*
N	----	----	15·97	16·03	----	----	*16·00*
S	- --	----	----	----	1·15	1·19	*1·17*
P	----	----	----	----	----	----	*0*
O	----	----	----	----	----	----	*23·99*
							100·00

solved in water and dialyzed until the greater portion of the salt was removed. The solution was then concentrated and the substance reprecipitated by saturating the neutral solution with ammonium sulphate. This precipitate, after solution in water, was then dialyzed until all of the ammonium sulphate was removed, after which the solution was concentrated and precipitated with alcohol. When dry, the substance gave by analysis the results shown in the accompanying table.

This body differs from all of the preceding preparations, in that it is not precipitated from an aqueous solution by acetic acid. Neither is it precipitated at all by the addition of salt in substance; but the addition of a little acetic acid to the salt-saturated fluid gives a heavy precipitate which, however, does not represent all of the deuterocaseose, since the filtrate gives an additional precipitate with ammonium sulphate. Apparently about one-half of the substance is precipitated by acetic acid. Further, the acetic acid precipitate in this case differs from the protocaseose precipitate with acid, in that it is readily and completely soluble in water. This body, therefore, which certainly must represent *pure* deuterocaseose, shows a close resemblance to the pure deuteroalbumose separated by Neumeister. Like the latter, it does not give any precipitate whatever with cupric sulphate nor with ferric chloride and only the faintest turbidity with acetic acid and potassium ferrocyanide.

D. *Heterocaseose.*

In each digestion, evidence was obtained at various points in the process of separation, noticeably on dialysis of the first protoalbumose precipitate, of the presence of a body insoluble in water but soluble in dilute sodium chloride solution. The quantity of the substance, however, was in most cases exceedingly small, so much so that nothing more than a few reactions could be tried with it. In the present digestion, however, the amount was somewhat larger, and sufficed for a partial analysis. The substance was obtained as a more or less gummy residue, on dialysis of protoalbumose 1. It was purified by solution in 10 per cent. sodium chloride and separation by dialysis. Like heteroalbumose, the whole of the substance was not now soluble in salt solution, for a portion had apparently been converted into a body resembling dysalbumose, insoluble in salt solution but soluble in 0·2 per cent. hydrochloric acid.

Analysis of the dried substance gave the following results:

I. 0·4011 gram substance gave 0·2463 gram H_2O=6·82 per cent. H and 0·7434 gram CO_2=50·54 per cent. C.

II. 0·4287 gram gave 53·6 c. c. N at 18·6° C. and 760·1 mm. pressure =14·70 per cent. N.

III. 0·4118 gram gave 0·0256 gram ash =6·21 per cent.

The ash-free substance would therefore contain

53·88 per cent. C,   7·27 per cent. H,   15·67 per cent. N.

Reactions of the caseoses.

Under this head little need be said. The reactions characteristic of the albumose bodies in general will apply here. Certain differences, however, have already appeared in our description of the processes incident to separation of the caseoses. Protocaseose, unlike protoalbumose, is precipitated from an *aqueous* solution by acetic acid. The precipitation, however, is not complete; saturation of the acid filtrate with sodium chloride, invariably gives an additional precipitate which is the heavier of the two. Further, long-continued washing of the acid precipitate with water or salt solution appears to partially remove the acid from the caseose body. Protocaseose is likewise precipitated from an aqueous solution by hydrochloric, nitric and sulphuric acids; the precipitate, however, is far less soluble in excess of sulphuric or nitric acid than in excess of the other two acids. In very dilute acids, protocaseose is soluble and is partially precipitated by addition of stronger acid of the same kind; thus the substance is readily soluble in 0·4 per cent. hydrochloric acid, from which solution it is precipitated by the addition of a little concentrated acid, this precipitate dissolving on the addition of more acid. Evidently then, protocaseose as fast as formed by the action of pepsin-hydrochloric acid, would dissolve in the acid gastric juice and not be mixed with the jelly-like insoluble residue. Boiled with dilute or strong acid, protocaseose is apparently not changed; at least no precipitate is obtained on neutralization of the acid fluid. The acetic acid solution of protocaseose gives a very heavy precipitate with potassium ferrocyanide. In an aqueous solution of the substance, cupric sulphate gives a heavy curd-like precipitate, while ferric chloride gives a similar precipitate readily soluble in excess of the precipitant. Like protoalbumose, protocaseose is precipitated by saturation of its aqueous solution with sodium chloride, but never completely; there always remains in the filtrate, a portion of the substance precipitable only on addition of acetic acid.

Of the several preparations of deuterocaseose, those precipitated by ammonium sulphate are evidently the only ones perfectly pure. Further, it is evident that this substance can be obtained pure only by complete removal of all protocaseose from the solution, which implies precipitation of a large portion of the deuterocaseose also, and then precipitation of the small amount of deutero remaining, by saturation of the fluid with ammonium sulphate. D deutero, prepared in this manner, shows several very marked points of difference from protocaseose. In the first place, it is not precipitated in an *aqueous* solution by acetic acid. Further, the addition of potassium ferrocyanide to a solution acidified with acetic acid gives no precipitate whatever. Cupric sulphate and ferric chloride both fail to produce any precipitate in an aqueous solution. Pure deuterocaseose, as already mentioned, is not precipitated by saturation of its aqueous solution with sodium chloride; addition of acetic acid, however, to the salt-saturated solution gives a heavy precipitate, which represents perhaps half of the deutero, the remainder of which is precipitated only by saturation of the fluid with ammonium sulphate. It is thus evident that in the precipitation of protocaseose from a salt-saturated solution by acetic acid, more or less deuterocaseose will be likewise precipitated, the amount depending probably on the concentration of the solution and other minor circumstances. Hence, A deutero is unquestionably contaminated with some protocaseose, and on the other hand protocaseose D 3 and 4, and perhaps protocaseose C 2, without doubt contain some deuterocaseose. That *A* deutero contains some protocaseose, is evident from the fact that it gives a precipitate with cupric sulphate, and further its aqueous solution is rendered decidedly turbid by acetic acid. Moreover, the protocaseose precipitated by acetic acid, and which may contain some deutero, appears to differ in one or two respects from either proto or deuterocaseose. Thus, an aqueous solution of the purified substance is precipitated like pure protocaseose by acetic acid, but the precipitate is only partially soluble in excess of the acid and even that requires a large excess.

With nitric acid, pure deuterocaseose gives no precipitate, but on warming the solution the xanthoprotein reaction comes out strongly.

Composition of the caseoses and their relation to casein.

In studying the composition of the various caseoses we have been hampered by the large percentage of ash invariably present in all of

TABLE SHOWING RELATIVE COMPOSITION OF THE CASEOSES.

Protocaseose.	C	H	N	S	O
A 1 NaCl precipitate	52·50	7·15	15·73	0·96	23·66
A 2 " "	53·85	7·21	15·84	0 98	22·12
A 3 Acetic acid precipitate	52·59	7·17	15·70	0·90	23·64
B 1 NaCl precipitate	52·91	7·06	15·65	0·90	23·48
B 2 Acetic acid precipitate	52·43	7·01	16·19	0·90	23·47
C 1 NaCl precipitate	52·64	7·08	...	....	....
C 2 Acetic acid precipitate	51·73	7·15	15·85	0·79	24·48
D 1 NaCl precipitate	53·93	7·17	16·05	0·85	22·00
D 2 " "	52·84	7·10	15·86	1·04	23·16
D 3 Acetic acid precipitate	52·05	7·13	16·12	1·06	23·64
D 4 " " "	52·88	7·07	16·13	0·98	22·94
Deuterocaseose.					
A. Acetic acid precipitate	51·59	6·98	15·73	0·75	25·03
D. $(NH_4)_2SO_4$ "	51·79	7·05	16·00	1·17	23·99
Heterocaseose.					
D.	53·88	7·27	15·67	....	....
Casein.					
Average of Nos. I–VII	53·30	7·07	15·91	0·82	22·03

the preparations. We have already commented on the difficulty, in fact, impossibility, of removing certain inorganic salts after they have once been brought in contact with a caseose body. Repeated precipitation appears to affect the percentage of ash but little. The reason for the large percentage of ash lies in the precipitation of the caseoses from such large volumes of fluid. We thought it unwise at first, to expose the bodies to the long continued evaporation necessary for precipitation with a small amount of salt. To avoid the possible danger of change, therefore, the large volumes of fluid resulting from the several digestions were saturated directly with salt, and as this involved the use of large quantities, calcium salts and some iron as impurities in the sodium chloride, were unavoidably introduced. These, the caseoses seemed at once to catch hold of and retain, in spite of oft-repeated purification. In the digestion *D*, in which the fluid was concentrated somewhat before precipitation, the percentage of ash is seen to be somewhat smaller than in preparations from the other digestions.

In comparing the composition of the individual protocaseoses (see the accompanying table) it is seen that two of the bodies show a content of carbon somewhat higher than casein itself, while the average of all the others, with one exception, shows a content of carbon a little lower than casein. Leaving out the acetic acid precipitate C 2, the average of the remaining ten preparations of protocaseose shows the following composition for this substance:

	C	*H*	*N*	*S*	*O*
Protocaseose	52·89	7·10	15·94	0·95	23·12
Casein	53·30	7·07	15·91	0·82	22·03

Plainly, the average of our results would indicate that protocaseose does not differ essentially in composition, from the casein from which it is formed. A slightly smaller content of carbon is the only noticeable difference. To be sure the individual results show noticeable variation in the percentage of carbon, but bearing in mind the large amount of ash present in the preparations, it is evident that the average result is of more value than the results obtained in any one case. As to the lower content of carbon in so-called protocaseose C 2, it is probable that this body is composed mainly of deuterocaseose. The two caseoses being precipitated together in this digestion by ammonium sulphate and then separated afterwards from a fairly concentrated solution by saturation with salt and addition of acetic acid, renders it probable that the protocaseose was more completely precipitated than usual by salt alone; and further, it is probable that on addition of acetic acid to the concentrated and salt-saturated fluid, a much larger proportion of deuterocaseose was precipitated. In confirmation of this view it was noticed that the amount of deuterocaseose obtained by the later precipitation with ammonium sulphate was quite small; far smaller proportionally than obtained in D. That the body contained some protocaseose, was evident from its reaction with cupric sulphate and with acetic acid.

Pure deuterocaseose evidently contains a smaller content of carbon than protocaseose. It is equally evident that it is a body further removed from casein than protocaseose. Its general reactions show a closer relationship to peptone than to casein or the proto-body. Heterocaseose, on the other hand, judging from analysis of a single preparation, contains fully as much if not more carbon than casein itself.

Nearly all of the caseoses show a somewhat higher percentage of sulphur than casein, but probably the increase (0·1 per cent.) is due mainly to a trace of sulphate in the ash, not accounted for. Owing to the large amount of phosphate in the ash of the different preparations, phosphorus was sought for only twice. In both of these, however (protocaseose D 1 and deuterocaseose D), the phosphorus in the ash was the exact equivalent of the total phosphorus found after fusion with potassium hydroxide and nitrate. This might indicate that in the cleavage of casein with pepsin-hydrochloric acid, the phosphorus of the casein is removed in the form of a phosphorized body, leaving the thus non-phosphorized matter to break down into the caseoses. With this thought in mind, we propose to study later the nature and composition of the insoluble, semi-gelatinous body separated in the first stage of digestion. We also hope to extend our work by a study of Weyl's commercial "casein-peptone," preliminary examination of which has shown us the presence in large quantities of caseoses. In this way and by a somewhat different method of isolating the individual caseoses, we hope to verify our present work and at the same time obtain products comparatively free from ash, with which to establish beyond question the composition of the caseoses. We are also occupied in a study of pure casein-peptone, purified according to the method made use of by Kühne and Chittenden in the study of fibrin-peptone.

XXIII.—INFLUENCE OF SOME ORGANIC AND INORGANIC SUBSTANCES ON GAS METABOLISM. BY R. H. CHITTENDEN AND G. W. CUMMINS, PH.B.

WHILE much time has been spent during the past few years in studying the influence of various substances on proteid metabolism, far less attention has been paid to the effects of these substances on the consumption of oxygen and the elimination of carbonic acid. Naturally in studying the influence of any substance on the nutrition of the body, we need to know not only its action on the excretion of nitrogen but also its influence on the production of carbonic acid. In this way only can we arrive at a true understanding of the influence of the substance on total metabolism, and obtain the necessary data from which to draw conclusions as to its influence on the consumption of either nitrogenous or non-nitrogenous matter. The difficulties, however, in the way of carrying on consecutive determinations of the relative amount of carbonic acid eliminated by the lungs are considerable, and in the absence of the necessary respiration apparatus, the difficulties are greatly increased. We have, however, endeavored to carry on some experiments in this direction, and although lacking the ordinary apparatus we have still been able with the means at our disposal to obtain some interesting results, a portion of which are simply confirmatory of previous work, while others are wholly new.

The apparatus employed in measuring the amount of carbonic acid eliminated is shown in the accompanying illustration (see Plate). The chamber in which the animal was placed during the experiment, was a bell jar of 32 litres capacity, with ground edge fitting closely upon a smooth glass plate. This when coated with grease made a perfectly tight joint, but in order to avoid any possibility of error, the jar and plate were placed in a shallow pan of galvanized iron, and water poured in to the depth of 2–3 inches, thus insuring a perfectly air-tight joint. In the top of the bell jar was an opening, closed with a doubly perforated rubber stopper, through which passed two tubes; one bringing air into the chamber, the other carrying it to the absorption apparatus. The inlet tube (to the left of the figure) was prolonged so as to admit the air nearly at the bottom of the jar, while the outlet tube came just through the stopper, thus insuring a perfect circulation of air. Air was drawn through the chamber by means of three aspirators, two of which had a capacity of 15 litres and one of 7½ litres. The three aspirators working together would therefore draw through the chamber 37½ litres of air at every filling, and the flow was so regulated that 30 minutes were required to draw that amount of air through the apparatus. The flow of water from the aspirators was quite regular, since the inlet tubes went to the bottom and the air had to bubble up through the water, as the latter ran out, on the principle of Mariotte's bottle. The rate of flow was regulated by carefully changing the difference in height between the inlet tube (for air) of the aspirator and the outlet tube (for water). This of course, at the outset, was a tedious operation, but when once perfected and the apparatus permanently set up, the three aspirators ran exactly together, with a maximum variation of 15 seconds for the half-hour, which variation, however, was seldom observed. In addition, each aspirator was marked off into eight divisions, the last one of which was equal to only one-half of the others. In the two large aspirators these divisions indicated exactly the same volume, while in the small aspirator the divisions represented half the capacity of the former; but the flow of water in the latter was regulated to consume the same amount of time as in the former. Hence four minutes were required for the water to flow by each of the first seven divisions, and two minutes for the last, making a total of thirty minutes for the entire volume of water to flow from each aspirator.

The tube drawing the respired air from the chamber in which the animal was enclosed, was divided a short distance from the chamber, as seen in the figure, and two-fifths of the mixed air was drawn successively through three absorption tubes filled with a standard solution of barium hydroxide for absorption of the carbonic acid. The absorption tubes were about two-thirds of a metre long and the lower tube (*a*) contained 100 c. c. of a standard baryta solution, the middle tube (*b*) also 100 c. c. of the solution, and the upper tube (*c*) 50 c. c. The amount of carbonic acid absorbed was, at the end of the experiment, determined by titration with a standard solution of oxalic acid, using phenol-thalein as an indicator. Two titrations were made, one of the contents of tube *a* and one of the contents of the two tubes *b* and *c*. By using the three tubes, absorption of the carbonic acid was quite complete. In order to aid absorption, the air was broken into small bubbles by being forced through a small tube dipping beneath the barium hydroxide. Frequent blank experiments showed that all of the connections were perfectly tight, and further, all of the tubes being in the same position, that the flow of water from each aspirator was perfectly uniform, and that the aspirators could be relied upon to draw the given volume of air through the apparatus in the time designated without any appreciable variation. In addition, the two-fifths drawn through the absorption tubes for determination of the carbonic acid was always exactly two-fifths of the aspirated air; since the aspirators, as already remarked, worked with perfect uniformity. Any tendency to variation, either in the time, or in the action of the individual aspirators, was noticed at the very outset of the experiment, as the water reached the level of the different marks on the aspirators, and could be at once checked or controlled by moving slightly the water outlet tube so as to either increase or diminish the difference in height between the latter and the inlet tube for air. Theoretically, variations in the temperature of the water in the aspirators might affect somewhat the volume of air analyzed, but a constant determination of the temperature of the water showed such slight variations that they did not seem to justify us in making any corrections for possible change in the amount of air aspirated. Naturally, all of the supports for the three absorption tubes were permanently placed, so that there could be no change of position; the tubes themselves placed in the same position in the holders; the volumes of baryta solution invariably the same, so as not to increase or decrease the pressure to be overcome; and lastly the aspirator tubes and stoppers fastened so as not to admit of any change. With these precautions, the results obtained, both as to the volume aspirated and the time consumed, were quite satisfactory.

As already mentioned, the total capacity of the three aspirators was 37½ litres or 5½ litres more than the capacity of the bell jar. This amount of air drawn through the chamber in 30 minutes, was more than enough to supply the largest rabbit experimented on, with the necessary amount of oxygen. But there must have been a slight accumulation of carbonic acid in the air of the chamber; this, however, was a constant factor throughout the experiments. Further, the results obtained, expressed in milligrams of CO_2, do not represent the total amount of carbonic acid eliminated by the rabbit during the thirty minutes of the experiment, but simply the amount of CO_2 contained in the 37½ litres of air aspirated during that time. Such a result, however, ought certainly to show just as plainly any influence on the elimination of carbonic acid, as a determination of absolute quantity and thus be equally valuable as an indication of influence or lack of influence on the gas metabolism of the body. Further, the results thus obtained ought to express equally as well, the comparative action of the various substances experimented with.

In every experiment, the time at which the animal was introduced into the bell jar was exactly noted and then two minutes were allowed before starting the aspirators, to make all of the connections properly. The rabbit was therefore under the bell jar, in each determination of carbonic acid, for exactly thirty-two minutes. The aspirators were started simultaneously and their progress carefully watched, in order to check any slight irregularity that might show itself.

The animals experimented with were wholly rabbits, and preliminary trials showed us plainly that it was very necessary to have them in a condition of hunger during the experiment, in order to avoid the irregularities incident to change in digestion. Further, we soon found that this was best accomplished by depriving the animal of food for three days, after which the experiment was commenced and allowed, as a rule, to extend through three consecutive days, the animal being deprived of food during the entire period. On the first of the three days, eight determinations of carbonic acid were made and the results obtained were used as a control, with which to compare the results obtained on the two following days, when the animal was being dosed with the substance experimented with. This, as a rule, we found to be the most satisfactory method of procedure, since small differences could not be relied upon as expressing anything of importance; for the varying restlessness of the confined animal, involving more or less muscular activity, would many times lead to variations in the amount of carbonic acid excreted, as may be noticed in the control experiments on those days when the animals were not dosed. Hence, the average of several consecutive results must necessarily express more correctly the average elimination of carbonic acid than any single result. Further, we deemed it better to allow the experiments to extend, as a rule, over several days and thus study the action of small, repeated doses of the various substances rather than to observe the effects of a single large dose, where violent action might naturally be expected.

The following table of results illustrates the way in which our experiments have been conducted, and at the same time shows the extent of variation, in the amount of carbonic acid, to be expected under normal circumstances from day to day. In this experiment, the rabbit had been deprived of food for three days, and the results show the amount of carbonic acid in the 37·5 litres of aspirated air for four distinct periods, during the fourth and fifth days. As already stated, the total amount of baryta solution employed in the three absorption tubes was 250 c. c., of which 100 c. c. were used in the first or lower tube (a), while the remainder was used in the two other tubes b and c. Several solutions of oxalic acid were employed, the average strength of which was such that 1 c. c. equaled about 20 milligrams of carbonic acid.

Time.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c c. $Ba(OH)_2$. c. c.	Difference. c c oxalic acid.	CO_2 in a, b and c mg.	CO_2 in 37·5 L. aspirated air. mg	Body temperature, ° C.
	tube a. c. c	tubes b and c. c. c.						
March 18.								
9:48 to 10:18	10·4	25·0	35·4	45·1	9·7	180·2	450·5	38·8
11:51 to 12:21	10·6	25·2	35·8	45·1	9·3	172·7	432·0	38·1
2:59 to 3:29	10·5	25·3	35·8	45·1	9·3	172·7	432·0	38·2
5:03 to 5:33	10·4	24·9	35·3	45·1	9·8	182·0	455·1	38·3
March 19.								
8:59 to 9:29	10·2	25·1	35·3	45·1	9·8	182·0	455·1	38·3
10:53 to 11:23	10·9	25·3	36·2	45·1	8·9	165·3	413·4	37·7
2:53 to 3:23	10·2	24·2	34·4	45·1	10·7	198·7	496·8	38·7
4:51 to 5:21	10·6	24·9	35·5	45·1	9·6	178·3	445·9	38·9
Average,	10·5	25·0	35·5	45·1	9·6	179·0	447·6	38·4

Action of uranyl nitrate.

As stated in a preceding article,* the physiological action of uranium salts has been little studied. Experiments are now in progress to show the influence of uranium on proteid metabolism, and our present results show the influence of this substance on the excretion of carbonic acid. The rabbit first experimented with was deprived of food for three days, and on the fourth day the experiment was commenced, extending through three entire days, during which time the animal was without food. The accompanying tables show the results obtained. The body temperature was ascertained by inserting a self-registering thermometer into the rectum.

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* Chittenden and Hutchinson, this volume.

A study of the first results shows plainly a decided action on the part of the uranium salt. The influence of the salt, however, manifests itself somewhat slowly, and it is not until the third day that its action becomes very pronounced, when the increased excretion of carbonic acid becomes very noticeable, accompanied with a slight rise in temperature. The first action of the uranium appears to cause a diminution in body temperature and in the amount of carbonic acid eliminated. The total amount of uranium salt given was quite large (1·175 grams in divided doses), and although no especial toxic symptoms showed themselves, the animal died on the day following the conclusion of the experiment.

A second series of experiments was tried, using smaller amounts of uranium nitrate and extending through four days, the results of which are also shown in the accompanying tables. The rabbit was deprived of food for four days prior to commencing the experiment. The amount of uranium nitrate given was considerably smaller than the quantity employed in the first series of experiments, and the animal did not suffer any permanent ill effects from its use. The following table shows the average daily result, expressed in milligrams of CO_2 contained in the 37·5 litres of aspirated air, together with the average body temperature.

May 3.	38·9° C.	574·3 milligrams CO_2
“ 4.	39·0	540·8 “ “
“ 5.	39·9	581·2 “ “
“ 6.	38·5	716·3 “ “

The uranium nitrate was introduced by hypodermic injection in the following quantities:

May 3.		0
“ 4.	5:18 p. m.	0·080 gram of the salt.
“ 5.	8:40 a. m.	0·090 “ “
“ 5.	10:20 a. m.	0·100 “ “
“ 5.	12:40 p. m.	0·150 “ “
“ 5.	3:25 p. m.	0·150 “ “
“ 5.	5:15 p. m.	0·200 “ “
“ 6.		0
		0·770

In this second series of experiments it is to be noticed that the first two days are given up wholly to determining the normal excretion of carbonic acid, and the results show fully how close an agreement may be expected under normal circumstances. Taking the results

FIRST SERIES OF EXPERIMENTS WITH URANIUM.

Normal period, without uranium nitrate.

Date.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used c c	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c oxalic acid.	CO_2 in a. b and c. mg	CO_2 in 37·5 L aspirated air. mg.	Body temperature. °C
April 19	tube a c. c	tubes b and c. c c						
A. M. 9:47 to 10:17	6·5	24·8	31·3	46·3	15·0	278·9	697·3	38·7
10:45 to 11:15	7·8	25·8	33·6	46·3	12·7	235·3	588·3	38·7
11:46 to 12:16	7·9	25·8	33·7	46·3	12·6	233·4	583·6	38·6
P. M. 2:04 to 2:34	8·4	26·3	34·7	46·3	11·6	214·9	537·3	38·4
2:57 to 3:27	8·4	26·1	34·5	46·3	11·8	218·6	546·6	38·7
3:58 to 4:28	8·2	26·1	34·3	46·3	12·0	221·4	553·5	38·6
4:52 to 5:22	8·5	26·2	34·7	46·3	11·6	214·9	537·3	38·7
Average,	7·9	25·9	33·8	46·3	12·5	231·0	577·7	38·9
April 20.			*With uranium nitrate.*					
A. M. 9:04 to 9:34	7·3	25·9	33·2	46·3	13·1	242·7	606·8	38·4
9:57 to 10:27	8·3	26·8	35·1	46·3	11·2	207·5	518·8	38·4
10:59 to 11:29	9·8	26·6	36·4	46·3	9·9	183·4	458·6	38·2
11:53 to 12:23	8·7	26·3	35·0	46·3	11·3	209·3	523·5	38·1
P. M. 1:59 to 2:29	8·8	26·3	35·1	46·3	11·2	207·5	518·8	38·4
2:51 to 3:21	8·8	26·5	35·3	46·3	11·0	204·8	512·0	38·8
3:46 to 4:16	8·4	26·1	34·5	46·3	11·8	218·6	446·6	39·1
4:41 to 5:11	6·8	25·2	32·0	46·3	14·3	264·9	662·4	38·9
Average,	8·4	26·2	34·6	46·3	11·7	217·3	535·9	38·5

With uranium nitrate—continued.

Date April 21.	Oxalic acid to neutralize baryta solution		Total oxalic acid used c c	Oxalic acid equivalent to 250 c c Ba(OH)$_2$. c c	Difference c c oxalic acid.	CO$_2$ in a b and c mg	CO$_2$ in 37·5 L aspirated air. mg.	Body temperature. C.
	tube a c c.	tubes b and c. c c						
A. M. 9:02 to 9:32	5·0	22·4	27·4	46·3	18·9	350·1	875·5	39·2
9:58 to 10:28	6·0	24·9	30·9	46·3	15·4	285·1	712·8	38·9
10:55 to 11:25	6·0	24·7	30·7	46·3	15·6	289·0	722·6	39·0
11·47 to 12:17	5·7	24·8	30·5	46·3	15·8	292·7	731·9	39·4
P M. 2:00 to 2:30	7·3	25·7	33·0	46·3	13·3	246·4	616·1	39·1
2:55 to 3:25	6·0	25·0	31·0	46·3	15·3	283·4	708·7	39·1
3:47 to 4:17	8·4	26·2	34·6	46·3	11·7	216·7	541·5	39·2
4:42 to 5:12	9·3	26·6	35·9	46·3	10·4	192·6	481·7	39·1
Average,	6·7	25·0	31·7	46·3	14·6	269·5	673·9	39·1

The following figures give the average daily result in body temperature and in the amount of carbonic acid contained in 37·5 litres of aspirated air:

April 19,	38·9° C.	577·7 milligrams CO$_2$		
" 20,	38·5 "	535·9	"	"
" 21,	39·1 "	673·9	"	"

The uranium nitrate was introduced by hypodermic injection in the following quantities:

April 19,	5·40 p. m.	0·050 gram of the salt.		
" 20,	8·55 a. m.	0·100	"	"
" 20,	10·35 a. m.	0·100	"	"
" 20,	12·40 p. m.	0·150	"	"
" 20,	1·35 p. m.	0·150	"	"
" 20,	5·30 p. m.	0·300	"	"
" 21,	8·55 a. m.	0·200	"	"
" 21,	2·45 p. m.	0·125	"	"
		1·175		

The animal died on the 22d.

SECOND SERIES OF EXPERIMENTS WITH URANIUM.

Normal period, without uranium.

Date.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c c	Oxalic acid equivalent to 250 c c. Ba(OH)$_2$ c c.	Difference. c c. oxalic acid	CO$_2$ in a, b and c. mg.	CO$_2$ in 37·5 L. aspirated air mg.	Body temperature. ° C
May 3.	tube a. c. c.	tubes b and c. c. c.						
A. M. 9:08 to 9:38	9·2	24·3	33·5	44·8	11·3	228·4	571·1	38·9
10:08 to 10:38	9·5	25·0	34·5	44·8	10·3	208·2	520·5	38·8
11:03 to 11:33	8·7	24·4	33·1	44·8	11·7	236·5	591·3	38·9
11:57 to 12:27	8·6	24·2	32·8	44·8	12·0	242·5	606·5	38·7
P. M. 2:07 to 2:37	9·7	25·0	34·7	44·8	10·1	204·1	510·4	38·8
2:59 to 3:29	8·3	24·2	32·5	44·8	12·3	248·6	621·6	38·9
3:50 to 4:20	8·7	24·5	33·2	44·8	11·6	234·5	586·3	38·9
4:44 to 5:14	8·7	24·5	33·2	44·8	11·6	234·5	586·3	39·4
Average,	8·9	24·5	33·4	44·8	11·4	229·7	574·3	38·9

May 4. *Normal period—continued.*

Date.	tube a. c. c.	tubes b and c. c. c.	Total oxalic acid used. c c	Oxalic acid equivalent to 250 c c. Ba(OH)$_2$ c c.	Difference. c c. oxalic acid	CO$_2$ in a, b and c. mg.	CO$_2$ in 37·5 L. aspirated air mg.	Body temperature. ° C
A. M. 8:50 to 9:20	9·2	24·7	33·9	44·8	10·9	220·3	550·9	38·9
9:49 to 10:19	9·5	24·4	33·9	44·8	10·9	220·3	550·9	38·9
10:44 to 11:14	8·9	24·6	33·5	44·8	11·3	228·4	571·1	38·9
11:37 to 12:07	9·5	24·8	34·3	44·8	10·5	212·2	530·6	38·9
P. M. 1:56 to 2:26	9·1	25·0	34·1	44·8	10·7	216·3	540·8	39·1
2:48 to 3:18	9·6	25·2	34·8	44·8	10·0	202·1	505·6	39·2
3:41 to 4:11	9·4	25·0	34·4	44·8	10·4	210·2	525·6	39·1
4:32 to 5:02	9·3	24·8	34·1	44·8	10·7	216·3	540·8	39·0
Average,	9·3	24·8	34·1	44·8	10·7	216·3	540·8	39·0

With uranium nitrate.

Date. May 5.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used c.c.	Oxalic acid equivalent to 250 c.c. Ba(OH)$_2$ c.c.	Difference c.c. oxalic acid	CO$_2$ in a, b and c mg	CO$_2$ in 37·5 L. aspirated air. mg	Body temperature °C
	tube a c.c.	tubes b and c c.c.						
A. M. 8:48 to 9:18	8·7	24·6	33·3	44·8	11·5	232·1	581·2	39·1
9:41 to 10:11	8·5	24·5	33·0	44·8	11·8	238·5	596·3	39·3
10:39 to 11:19	9·0	24·8	33·8	44·8	11·0	222·3	555·9	39·6
11:34 to 12:04	8·7	24·6	33·3	44·8	11·5	232·4	581·2	39·9
P. M. 1:57 to 2:27	8·5	24·7	33·2	44·8	11·6	234·1	586·2	40·0
2:49 to 3:19	9·0	24·8	33·8	44·8	11·0	222·3	555·9	40·0
3:45 to 4:15	8·4	24·6	33·0	44·8	11·8	238·5	596·3	40·4
4:40 to 5:10	8·6	24·4	33·0	44·8	11·8	238·5	596·3	40·5
Average,	8·7	24·6	33·3	44·8	11·5	232·4	581·2	39·9

May 6. *With uranium nitrate—continued.*

A. M. 8:52 to 9:22	7·0	22·1	29·1	44·8	15·7	317·3	793·4	40·0
9:52 to 10:22	5·6	23·3	28·9	44·8	15·9	321·4	803·5	39·6
10:48 to 11:18	6·9	24·0	30·9	44·8	13·9	280·9	702·5	38·2
11:43 to 12:13	6·9	24·0	30·9	44·8	13·9	280·9	702·5	37·9
P. M. 2:11 to 2:41	7·5	24·6	32·1	44·8	12·7	256·7	641·8	37·4
3:02 to 3:32	7·5	24·4	31·9	44·8	12·9	261·7	654·4	38·1
Average,	6·9	23·7	30·6	44·8	14·2	286·5	716·3	38·5

EXPERIMENT WITH CUPRIC SULPHATE.

Normal period, without copper.

Date. May 10.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. Ba(OH)$_2$. c. c.	Difference. c. c. oxalic acid.	CO$_2$ in a, b and c. mg.	CO$_2$ in 37·5 L. aspirated air. mg.	Body temperature. °C.
	tube a c. c.	tubes b and c. c. c.						
A. M. 9:05 to 9:35	11·3	27·7	39·0	47·7	8·7	175·8	439·7	37·9
10:00 to 10:30	12·3	28·0	40·3	47·7	7·4	149·5	374·0	37·8
10:57 to 11:27	11·8	27·8	39·6	47·7	8·1	163·7	409·4	37·8
11:49 to 12:19	12·4	28·0	40·4	47·7	7·3	147·5	368·9	37·8
P. M. 2:07 to 2:37	12·2	27·9	40·1	47·7	7·6	153·6	384·1	37·8
3:00 to 3:30	11·9	28·0	39·9	47·7	7·8	157·6	394·2	37·7
3:52 to 4:22	12·4	28·0	40·4	47·7	7·3	147·5	368·9	37·8
4:42 to 5:12	12·5	27·9	40·4	47·7	7·3	146·5	366·4	38·3
Average,	12·1	27·9	40·0	47·7	7·7	155·2	388·2	37·9
May 11.	*With cupric sulphate.*							
A. M. 9:04 to 9:34	13·0	28·3	41·3	47·7	6·4	129·3	323·4	37·2
10:08 to 10:38	12·0	28·0	40·0	47·7	7·7	155·6	389·1	37·3
10:58 to 11:28	12·7	28·0	40·7	47·7	7·0	141·5	353·8	38·2
11:54 to 12:24	12·5	27·8	40·3	47·7	7·4	148·5	371·5	38·3
P. M. 1:56 to 2:26	11·8	27·9	39·7	47·7	8·0	161·7	404·3	36·7
2:49 to 3:19	13·0	28·2	41·2	47·7	6·5	131·4	328·5	35·7
3:43 to 4:13	13·2	28·2	41·4	47·7	6·3	127·3	318·4	36·6
4:39 to 5:09	13·7	28·3	42·0	47·7	5·7	115·2	288·1	35·8
Average,	12·7	28·1	40·8	47·7	6·9	138·8	347·1	37·0

With cupric sulphate—Continued.

Date. May 12	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c c	Oxalic acid equivalent to 250 c c. Ba(OH)$_2$. c. c	Difference c c. oxalic acid.	CO$_2$ in a b, and c mg.	CO$_2$ in 37·5 L. aspirated air mg.	Body temperature ° C.
	tube a c c	tubes b and c c. c						
A. M. 9:11 to 9:41	13·3	28·3	41·6	47·7	6·1	123·3	308·3	35·2
10:06 to 10:36	13·6	28·3	41·9	47·7	5·8	117·2	293·1	35·3
11:00 to 11:30	14·7	28·3	43·0	47·7	4·7	95·0	237·5	35·7
11:52 to 12:22	13·6	28·4	42·0	47·7	5·7	115·2	288·1	35·8
P. M. 2:15 to 2:45	13·7	28·2	41·9	47·7	5·8	117·2	293·1	35·9
3:09 to 3:39	13·7	28·3	42·0	47·7	5·7	114·2	285·5	36·1
3:59 to 4:29	14·4	28·3	42·7	47·7	5·0	101·0	252.7	36·2
4.53 to 5:23	14·0	28·4	42·4	47·7	5·3	107·1	267·9	36·2
Average,	13·9	28·3	42·2	47·7	5·5	111·3	278·3	35·8

Following are the average daily results, expressed in milligrams of CO$_2$ contained in 37·5 litres of aspirated air, together with the average body temperature:

May 10.	37·9° C.	388·2 milligrams CO$_2$
" 11.	37·0	347·1 " "
" 12.	35·8	278·3 " "

The cupric sulphate was introduced by hypodermic injection, in the following amounts:

May 10.	5·34 p. m.	0·025 gram CuSO$_4$
" 11.	8:57 a. m.	0·005 " "
" 11.	9:55 a. m.	0·025 " "
" 11.	12:20 p. m.	0·050 " "
" 12.	9:08 a. m.	0·025 " "
		0·130

Rabbit died on the 13th.

of these two days for comparison, it is seen that the action of the uranium is somewhat slow, but that it produces on the first day (May 5) a noticeable rise in temperature, without any appreciable change in the elimination of carbonic acid. The full effect of the uranium, however, shows itself on the day following the last dose of the salt, and we then see the same noticeable increase in the elimination of carbonic acid noticed in the first series of experiments. We have to conclude, then, that uranium nitrate, when taken in sufficient quantity, tends to raise materially the body temperature and to increase very noticeably the excretion of carbonic acid.

Action of cupric sulphate.

Falck, as quoted by Dr. H. C Wood,* has found that cupric sulphate acts upon dogs, pigeons, rabbits and similar animals as an irritant, neurotic poison; producing great depression of temperature, with progressive general paresis ending in death, apparently from failure of respiration. Our experiments on rabbits show a marked influence of the salt in depressing body temperature and a still greater influence in diminishing the production of carbonic acid. The results of one experiment are shown in the preceding table. Although but 130 milligrams of the copper salt were used altogether, the animal finally died on the day following the conclusion of the experiment.

Action of arsenious oxide.

C. Schmidt and Stürzwage† have shown by experiments on cats, that arsenious acid tends to produce a noticeable diminution in the excretion of both nitrogen and carbonic acid. Voit, however, has pointed out that in these experiments, the diminished excretion depends simply on the loss of a large portion of the food by vomiting, and Bolck‡ has shown that small doses of arsenious oxide given to hungry dogs, is wholly without influence on the decomposition of proteid matter. With large, toxic doses of arsenic, Gähtgens§ and Kossel‖ have shown that a very noticeable increase in the elimination of nitrogen takes place. These facts constitute about the sum total of our knowledge regarding the action of arsenic on tissue changes.

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* Therapeutics, Materia Medica and Toxicology, p. 46.

† Moleschott's Untersuchungen, vi, p. 283.

‡ Zeitschrift für Biologie, vii, p. 430.

§ Centralblatt f. Med. Wissen., 1875, p. 529.

‖ Archiv. f. exper. Path. u. Pharm., v, p. 128.

Our experiments were made with rabbits in a condition of hunger, deprived of food for three days prior to the experiment, and the results appear to show that arsenious acid, in the case of rabbits, has a tendency to diminish the excretion of carbonic acid, presumably through its action on the metabolic activity of the tissue cells. The amount of arsenic given was quite small and the animal seemed wholly unaffected by the poison.

Action of potassium antimony tartrate.

Voit* states that antimony in large doses affects proteid metabolism in the same manner as arsenic, and since Saikowsky has shown that both arsenic and antimony tend to produce a fatty degeneration of the various organs, in which presumably the fat is formed from the decomposition of proteid matter, the non-nitrogenous moiety of the albumin molecule being stored up as fat instead of being burned to carbonic acid, it seems natural to expect that these two substances when taken in large quantity at least, should like phosphorus diminish both the consumption of oxygen and the elimination of carbonic acid.

With rabbits our results with antimony certainly lead to this conclusion. Even small doses of tartar emetic quickly lead to a diminished excretion of carbonic acid and also to a noticeable fall in temperature. In the first series of experiments, the results of which are shown in the accompanying tables, the excretion of carbonic acid fell from 363·6 milligrams per 37·5 litres of aspirated air to 203·8 milligrams and with a fall in temperature of from 39° C. to 34·6° C. The total amount of tartar emetic given was 82 milligrams.

In the second series of experiments, where as before, the rabbit had been deprived of food for three days prior to the experiment, still smaller quantities of antimony were given with even more pronounced results, both in the diminution of carbonic acid and in the depression of temperature. Thus while in the normal period the excretion of carbonic acid amounted to 396 milligrams per 37·5 litres of aspirated air and with a normal temperature of 38·6° C., tartar emetic (0·055 gram) given in divided doses reduced the carbonic acid to 106·5 milligrams per 37·5 litres of aspirated air and the temperature to 27·0° C. Ackermann† has already called attention to the great decrease in animal heat produced by antimony, notably in the case of rabbits.

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* Hermann's Handbuch der Physiologie, Band vi, p. 184.

† See H. C. Wood, Therapeutics, etc., p. 158.

EXPERIMENT WITH ARSENIOUS OXIDE.

Normal period, without arsenic.

Date.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$ c. c.	Difference c. c. oxalic acid	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. respirated air mg.	Body temperature. °C.
June 7	tube a c. c.	tubes b and c. c. c.						
A. M. 10:04 to 10:34	7·8	24·5	32·3	44·3	12·0	242·5	606·5	38·7
11:08 to 11:38	7·5	25·2	32·7	45·5	12·8	258·7	646·9	38·9
12:06 to 12:36	9·2	26·2	35·4	45·5	10·1	204·1	510·4	38·9
P. M. 1:57 to 2:27	8·3	25·6	33·9	45·5	11·6	234·5	586·3	38·9
2:52 to 3:22	8·6	25·7	34·3	45·5	11·2	226·4	566·0	39·0
3:50 to 4:20	6·9	25·5	32·4	45·5	13.1	264·8	662·0	39·3
4:44 to 5:14	9·1	24·9	34·0	45·5	10·5	211·2	528·1	38·9
Average,	8·2	25·4	33·6	45·3	11·6	234·6	586·6	38·9
June 8.	*With arsenious oxide.*							
A. M. 9:00 to 9:30	9·0	25·8	34·8	45·5	10·7	215·2	538·2	38·8
9:52 to 10:22	10·3	26·4	36·7	45·5	8·8	177·8	444·7	38·7
10:45 to 11:15	10·1	26·3	36·4	45·5	9·1	183·9	459·9	38·8
11:37 to 12:07	9·8	26·4	36·2	45·5	9·3	188·0	470·0	38·8
P. M.* 2:14 to 2:44	9·1	26·0	35·1	45·5	10·4	218·3	545·8	39·0
3:08 to 3:38	10·4	26·5	36·9	45·5	8·6	172·8	432·1	39·0
3:58 to 4:28	10·0	26·4	36·4	45·5	9·1	182·9	457·4	39·2
4:48 to 5:18	9·4	26·3	35·7	45·5	9·8	198·1	495·3	39·2
Average,	9·8	26·3	36·0	45·5	9·5	192·1	480·4	38·9

With arsenious oxide—continued.

Date June 9.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c.c.	Oxalic acid equivalent to 250 c.c. Ba(OH)$_2$. c.c.	Difference c.c. oxalic acid.	CO$_2$ in a. b and c. mg.	CO$_2$ in 37·5 L. aspirated air. mg.	Body temperature. °C.
	tube a. c.c.	tubes b and c. c.c.						
A. M. 9:00 to 9:30	9·4	26·1	35·5	45·5	10·0	202·1	505·4	39·0
9:52 to 10:22	10·4	26·5	36·9	45·5	8·6	172·8	432·1	38·9
10:41 to 11:11	9·1	26·1	35·2	45·5	10·3	208·2	520·5	39·1
11:36 to 12:06	9·6	26·3	35·9	45·5	9·6	194·0	485·2	39·0
P. M. 2:15 to 2:45	9·6	26·5	36·1	45·5	9·4	190·0	475·1	39·2
3:08 to 3:38	9·6	26·5	36·1	45·5	9·4	190·0	475·1	38·9
3:57 to 4:27	10·3	26·5	36·8	45·5	8·7	175·8	439·7	39·2
4:47 to 5:17	10·0	26·5	36·5	45·5	9·0	181·9	454·8	39·2
Average,	9·7	26·4	36·1	45·5	9·4	189·3	473·5	39·1

Average daily excretion of carbonic acid expressed in milligrams of CO$_2$ per 37·5 litres of aspirated air, and average temperature is as follows :

June 7	38·9° C.	586·6 milligrams CO$_2$
" 8	38·9	480·4 " "
" 9	39·1	473·5 " "

The arsenious oxide was introduced by way of the mouth in small gelatin capsules, in the following doses :

June 7	5:25 p. m.	0·005 gram	As$_2$O$_3$
" 8	8:47 a. m.	0·005 "	"
" 8	12:12 p. m.	0·005 "	"
" 8	5:25 p. m.	0·005 "	"
" 9	8:50 a. m.	0·005 "	"
" 9	12:12 p. m.	0·010 "	"
		0·035	

FIRST SERIES OF EXPERIMENTS WITH ANTIMONY.

Normal period, without tartar emetic.

Date. March 31.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c c	Oxalic acid equivalent to 250 c c Ba(OH)₂ c c	Difference c. c oxalic acid.	CO₂ in a b and c mg	CO₂ in 37·5 L aspirated air.	Body temperature °C.
	tube a. c c.	tubes b and c c c						
A. M.								
9:03 to 9:33	13·9	30·9	44·8	52·6	7·8	144·5	361·3	38·6
10:01 to 10:31	13·8	31·0	44·8	52·6	7·8	144.5	361·3	38·8
10:56 to 11:26	15·0	31·0	46·0	52·6	6·6	122·2	305·7	38·9
11:52 to 12:22	13·7	30·8	44·5	52 6	8·1	150·0	375·2	39·2
P. M								
1:55 to 2:25	12·5	30·0	42·5	52·6	10·1	187·0	467·7	39·1
2:47 to 3:17	15·0	31·1	46·1	52 6	6·5	120·4	301·1	39·2
3:41 to 4:11	14·4	30·4	44·8	52·6	7·8	144·5	361·3	30·2
4:36 to 5:06	13·7	30·8	44·5	52·6	8·1	150·0	375·2	39·3
Average,	14·0	30·7	44·7	52·6	7.85	145·4	363·6	39·0
April 1.			*With tartar emetic.*					
A. M.								
8:57 to 9:27	14·1	30·8	44·9	52·6	7·7	141·7	354·4	38·1
9:54 to 10:24	14·5	31·0	45·5	52·6	7·1	131·5	328·9	36·9
10:50 to 11:20	15·4	31·0	46·4	52·6	6·2	114·8	287·2	36·4
11:44 to 12:24	15·4	31·0	46·4	52·6	6·2	114·8	287·2	35·7
P. M								
2:01 to 2:31	17·0	31·2	48·2	52·6	4·4	81·5	203·8	34·6
Average,	15·3	31·0	46·3	52·6	6·3	116·9	292·3	36·3

The antimony was given in the form of tartar emetic and was introduced by hypodermic injection as follows:

March 31.	5:20 p. m.	0·012 gram tartar emetic.
April 1.	8:45 a. m.	0·035 " "
" 1.	12:43 p. m.	0·035 " "
		0·082

Rabbit died at 3:30 p. m., April 1.

SECOND SERIES OF EXPERIMENTS WITH ANTIMONY.

Normal period, without tartar emetic.

Date. April 5.	Oxalic acid to neutralize baryta solution: tube a c. c.	Oxalic acid to neutralize baryta solution: tubes b and c c. c.	Total oxalic acid used c. c.	Oxalic acid equivalent to 250 c. c. Ba(OH)$_2$ c. c.	Difference c. c. oxalic acid	CO$_2$ in a, b and c mg.	CO$_2$ in 37·5 L. aspirated air	Body temperature °C.
A. M. 9:14 to 9:44	14·4	30·9	45·3	52·6	7·3	135·2	338·1	38·4
10:09 to 10:39	13·0	30·3	43·3	52·6	9·3	172·3	430·8	38·5
11:04 to 11:34	13·1	30·5	43·6	52·6	9·0	166·7	416·9	38·4
11:57 to 12:27	14·5	30·8	45·3	52·6	7·3	135·2	338·1	38·6
P. M. 2:05 to 2:35	13·8	30·7	44·5	52·6	8·1	150·0	375·2	38·9
2:58 to 3:28	13·1	30·5	43·6	52·6	9·0	166·7	416·9	38·8
3:52 to 4:22	12·7	30·5	43·2	52·6	9·4	174·1	435·4	38·9
4:45 to 5:15	12·9	30·7	43·6	52·6	9·0	166·7	416·9	38·7
Average,	13·4	30·6	44·0	52·6	8·5	158·4	396·0	38·6
April 6			*With tartar emetic.*					
A. M. 8:59 to 9:29	14·5	30·8	45·3	52·6	7·3	135·2	338·1	37·4
9:57 to 10:27	15·2	31·1	46·3	52·6	6·3	116·7	291·8	36·7
10:52 to 11:22	14·5	30·6	45·1	52·6	7·5	138·9	347·4	36·6
11:46 to 12:16	14·5	31·0	45·5	52·6	7·1	131·5	328·9	37·3
P. M. 1:58 to 2:28	15·3	30·9	46·2	52·6	6·35	117·6	294·1	36·2
2:50 to 3:20	14·5	30·9	45·4	52·6	7·2	133·4	333·5	36·2
3:42 to 4:12	15·4	31·2	46·6	52·6	5·95	110·2	275·6	35·4
4:38 to 5:08	15·7	31·2	46·9	52·6	5·7	115·6	289·0	35·7
Average,	14·9	31·0	45·9	52·6	6·68	124·9	312·2	36·7

With tartar emetic—continued.

Date April 7	Oxalic acid to neutralize baryta solution		Total oxalic acid used c c	Oxalic acid equivalent to 250 c c $Ba(OH)_2$ c c	Difference c c oxalic acid	CO_2 in a, b and c mg	CO_2 in 37·5 L aspirated air mg	Body temperature °C
	tube a c. c	tubes b and c c c						
A M 9.00 to 9:30	17·6	31·2	48·8	52·6	3·8	70·4	176·0	30·0
9:59 to 10:29	18·5	31·3	49·8	52·6	2·8	51·8	129·7	28·0
10:53 to 11:23	18·9	31·4	50·3	52·6	2·3	42·6	106·5	27·0
Average,	18·3	31·3	49·6	52·6	2·97	54·9	137·4	28·3

Average daily excretion of carbonic acid expressed in milligrams of CO_2 per 37·5 litres of aspirated air, together with average temperature is as follows :

April 5.	38·6° C.	396·0 milligrams CO_2
6.	36·7	312·0 “ “
7.	28·3	137·4 “ “

The following amounts of antimony were injected :

April 5.	5:30 p. m.	0·015 gram tartar emetic.
6.	8:45 a. m.	0·015 “ “
6.	12:39 p. m.	0·015 “ “
6.	5:24 p. m.	0·010 “ “
		0·055

Rabbit died at 12 m., April 7.

Action of morphine sulphate.

Boeck and Bauer* have already made a careful study of the action of morphine on the elimination of carbonic acid and the absorption of oxygen. By experiments on a cat and on a dog they found that the action of morphine on metabolism was mainly an indirect one, affecting especially the consumption of non-nitrogenous matter. Further, that its action hinged mainly on its power of affecting muscular activity; thus in the case of a cat the first action of morphine was to increase the elimination of carbonic acid and the consumption of oxygen, due to the increased muscular activity induced by the poison, while in the case of a dog, where narcosis was half induced, there was a diminution in the amount of carbonic acid eliminated amounting in one case to 27 per cent. This diminished excretion was due almost wholly to the quieting action of the morphine and was followed by an after period in which there was increased production of carbonic acid, due to the increased activity of the muscle tissue.

In these experiments the dose of morphine was 0·05 gram, in the form of chloride, and was introduced by subcutaneous injection. The injection of the poison was followed soon after by convulsions, etc., indicating vigorous toxic action. In our first series of experiments we endeavored to have the toxic action less pronounced, and for this reason the morphine was introduced by way of the mouth in repeated doses, the experiment extending through three days and into the fourth. The rabbit was deprived of food through the entire period and had also been kept without food for three days prior to the experiment. The data are to be found in the accompanying tables.

The results do not show any very marked action, either on the excretion of carbonic acid or on the body temperature. At no time was there any noticeable indication of increased muscular activity, the rabbit remaining fairly quiet in the chamber and showing no symptoms of tetanic convulsions. On the other hand there was no very profound narcotism. A study of the individual results, however, shows that directly after each dose of morphine, the excretion of carbonic acid fell quite noticeably for one or two periods. Such action as was produced, therefore, in this experiment, is to be considered simply as incidental to the semi-somnolent condition of the animal.

In a second shorter series of experiments with a rabbit, one single

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* Zeitschrift fur Biologie, Band x, p. 339.

First Series of Experiments with Morphine.

Normal period, without morphine.

Date. March 24.	Oxalic acid to neutralize baryta solution. tube a. c c.	tubes b and c c c.	Total oxalic acid used. c. c	Oxalic acid equivalent to 250 c. c Ba(OH)$_2$. c. c.	Difference c. c. oxalic acid.	CO$_2$ in a, b and c mg.	CO$_2$ in 37·5 L. aspirated air. mg.	Body temperature. ° C.
A. M.								
8:54 to 9:24	11.0	25 5	36·5	45·1	8·6	159·8	399·5	37·9
9:53 to 10:23	11·9	25·8	37·7	45·1	7·4	137·5	344·0	38·0
10:50 to 11:20	11·6	25·7	37·3	45·1	7·8	144·9	362·5	37·9
11:46 to 12:16	----	---	36·8	45·1	8·3	154·2	385·7	38·0
P. M.								
2:14 to 2:44	10·6	26·1	36·7	45·1	8·4	156·1	390·3	38·2
3:08 to 3:38	10·2	26·1	36·3	45·1	8·8	163·5	408·8	38·3
4:02 to 4:32	10·3	26·1	36·4	45·1	8·7	161·6	404·2	38·2
4:55 to 5:25	11·4	26·1	37·5	45·1	7·6	141·2	353·2	37·8
Average,	11·0	25·9	36·9	45·1	8·2	152·4	381·0	38·0
March 25.	*With morphine sulphate.*							
A. M.								
8:51 to 9:21	11·7	26·5	38·2	45·1	6·9	128·3	320·8	38·2
9:53 to 10:23	11·8	26·5	38·3	45·1	6·8	126·4	316·2	37·8
10:47 to 11:17	10:9	26·2	37·1	45·1	8·0	148·7	371·8	38·0
11:44 to 12:14	11·1	26·4	37·5	45·1	7·6	141·2	353·2	38·0
P. M.								
2:01 to 2:31	11·1	26·2	37·3	45·1	7.8	144·0	360·1	38·7
3:08 to 3:38	11·7	26·2	37·9	45·1	7·2	133·8	334·7	38·7
4:04 to 4:34	11·0	25·7	36·7	45·1	8·4	156·1	390·3	38·6
4:57 to 5:27	10·0	25·2	35·2	45·1	9·9	184·9	462·3	38·8
Average,	11·1	26·1	37·2	45·1	7·9	145·4	363·7	38.4

With morphine sulphate—continued.

Date. March 26.	Oxalic acid to neutralize baryta solution		Total oxalic acid used c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$ c. c.	Difference c. c. oxalic acid	CO_2 in a, b and c. mg.	CO_2 in 37·5 L. aspirated air. mg	Body temperature. ° C.
	tube a. c. c.	tubes b and c c. c.						
A. M. 9:24 to 9:54	17·6	35·8	53·4	60·5	7·1	131·5	328·9	38·8
10:19 to 10:49	16·4	35·6	52·0	60·5	8·5	157·4	393·7	38·8
11:14 to 11:44	16·5	35·7	52·2	60·5	8·3	153·7	384·5	38·6
12:09 to 12:39	16·5	35·7	53·2	60·5	8·3	153·7	384·5	38·9
P. M. 1:58 to 2:28	17·4	35·8	53·2	60·5	7·3	135·2	338·1	38·9
2:55 to 3:25	17·0	35·7	52·7	60·5	7·8	144·5	361·3	38·7
3:49 to 4:19	16·1	35·5	51·6	60·5	8·9	164·9	412·3	38·9
4:48 to 5:18	16·0	35·3	51·3	60·5	9·2	170·4	423·7	38·8
Average,	16·7	35·6	52·3	60·5	8·2	151·3	378·4	38·8
March 27.								
A. M. 8:33 to 9:03	16·2	35·6	51·8	60·5	8·7	161·1	403·0	38·9
9:30 to 10:00	15·7	35·5	51·2	60·5	9·3	172·3	430·8	38·9

Following is the average daily excretion of carbonic acid, expressed in milligrams per 37·5 litres of aspirated air, together with the average body temperature:

March 24.	38·0° C.	381·0 milligrams CO_2
" 25.	38·4	363·7 " "
" 26.	38·8	378·4 " "
" 27.	38·9	416·9 " "

The morphine was introduced into the stomach in solution in the following amounts:

March 24.	6:00 p. m.	0·075 gram morphine sulphate.
" 25.	8:35 a. m.	0·075 " "
" 25.	12:35 p. m.	0·075 " "
" 25.	5:50 p. m.	0·100 " "
" 26.	9:15 a. m.	0·100 " "
" 26.	12:50 p. m.	0·100 " "
		0·525

large dose of morphine sulphate was given, and given by hypodermic injection. In this case, as before, there was no increased muscular activity, but there was a sudden and rapid fall both in temperature and in the amount of carbonic acid excreted, the latter of which was still quite pronounced on the following day, although the temperature has gone back to normal. This diminution in carbonic acid was accompanied by a profound narcotism, the animal lying almost motionless and with a respiration ranging from 8 to 20 per minute. The results, which are to a certain extent corroborative of Boeck and Bauer's, are shown in the accompanying table:

SECOND SERIES OF EXPERIMENTS WITH MORPHINE.

Normal period without morphine sulphate.

Time.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. Ba(OH)$_2$. c c.	Difference c c. oxalic acid.	CO$_2$ in a, b and c mg	CO$_2$ in 37·5 L. aspirated air mg	Body temperature. °C.
	tube a. c. c.	tubes b and c. c c						
March 12. A. M. 9:12 to 9:42	15.4	34·1	49·5	59·25	9·75	180·6	465·0	39·1
10:00 A. M., injected subcutaneously 0·100 gram morphine sulphate.								
A. M. 10:23 to 10:53	16·9	34·5	51·4	59·25	7·85	145·4	363·5	36·6
11:29 to 11:59	17·3	34·5	51·8	59·25	7·45	138·0	342·5	34·4
12:32 to 1:02	16·3	34·1	50·4	59·25	8·85	164·0	410·0	33·3
P. M. 2:48 to 3:18	17·0	34·2	51·2	59·25	8·05	149·2	373·0	34·4
3:49 to 4:19	17·8	34·6	52·4	59·25	6·85	126·9	317·3	35·0
4:47 to 5:17	17·1	34·3	51·4	59·25	7·85	145·4	363·5	36·3
March 13. 10:08 to 10·38	16·9	34·3	51·2	59·25	8·05	149·2	373·0	39·2

Action of quinine sulphate.

The importance of quinine as a therapeutic agent and particularly its value as a febrifuge has led to a thorough study of its physiological action. In this connection, the action of quinine on proteid metabolism has been very thoroughly investigated, but as to its exact influence on the decomposition of non-nitrogenous matter, as shown by its effects on the elimination of carbonic acid, there is less unanimity of opinion. This is naturally a point of considerable importance for if, as is generally supposed, the alkaloid has the power of diminishing body temperature, it would presumably be due to its influence on the combustion of non-nitrogenous matter in the body. Ranke, Kerner, von Boeck and others have plainly shown the power of quinine to diminish proteid metabolism, but Strassburg, by an elaborate series of experiments* found that the alkaloid had no very decided effect upon the elimination of carbonic acid, either in healthy or fevered rabbits.

Boeck and Bauer,† however, from experiments on cats, claim that quinine in the first stage of its action diminishes somewhat the production of carbonic acid, owing to its inhibitory action on the tissue cells; but when large doses of quinine are given, so that convulsions appear, then there is an increased production of carbonic acid, owing to the greater decomposition of non-nitrogenous matter incident to increased muscular activity. With small doses of the alkaloid, it is to be presumed that the slight diminution in carbonic acid noticed by Boeck and Bauer comes simply from diminished proteid metabolism.

In our experiments, rabbits only were used and these in a condition of hunger, having been deprived of food for three days prior to the experiment. In the first series of experiments, the total amount of quinine given was quite large, so that at last the animal finally died from its effects. No decided action on the production of carbonic acid was noticed until just before the animal's death, when both the body temperature and the amount of carbonic acid fell quite noticeably. On the second day of the experiment, when the quinine was first being given, the body temperature, as taken per rectum, fell quite gradually until it finally reached a point 1·5 °C. below the average of the normal period. The results of the experiment are to be seen in the accompanying tables. The quinine given was in the form of hard, gelatin-coated pills and possibly was not as rapidly absorbed as might otherwise have been. At no time was the rabbit in convulsions.

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*Quoted from Dr. H. C. Wood, Therapeutics, p. 75.

† Zeitschrift für Biologie, Band x, p. 350.

FIRST SERIES OF EXPERIMENTS WITH QUININE.

Normal period, without quinine sulphate.

Date. April 12.	Oxalic acid to neutralize baryta solution		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$ c. c	Difference. c. c. oxalic acid.	CO_2 in a. b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.
	tube a. c c.	tubes b and c. c c.					
A. M. 9:11 to 9:41	9·5	24·2	33·7	41·5	7·8	144·5	361·5
10:05 to 10:35	10·8	24·4	35·2	41·5	6·3	116·7	291·8
10:58 to 11:28	10·8	24·2	35·0	41·5	6·5	120·4	301·1
11:54 to 12:24	10·7	24·2	34·9	41·5	6·6	122·2	305·7
P. M. 1:58 to 2:28	10·0	24·0	34·0	41·5	7·5	138·9	347·4
2:51 to 3:21	10·0	24·2	34·2	41·5	7·3	135·2	338·1
3:49 to 4:19	10·5	24·3	34·8	41·5	6·7	124·1	310·4
4:41 to 5:11	11·0	24·3	35·3	41·5	6·2	114·8	287·2
Average,	10·4	24·2	34·6	41·5	6·8	127·1	317·8

April 13. *With quinine sulphate.*

A. M. 9:14 to 9:44	9·3	23·9	33·2	41·5	8·3	153·7	384·5
10:10 to 10:40	11·3	23·9	35·2	41·5	6·3	116·7	291·8
11:05 to 11:35	11·0	24·4	35·4	41·5	6·1	113·0	282·6
11:58 to 12:28	10·3	24·2	34·5	41·5	7·0	129·7	314·3
P. M. 1:55 to 2:25	9·4	24·0	33·4	41·5	8·1	150·0	375·2
2:46 to 3:16	10·1	24·2	34·3	41·5	7·2	133·4	333·3
3:40 to 4:10	9·3	23·8	33·1	41·5	8·4	168·7	340·5
4:32 to 5:02	10·3	24·1	34·4	41·5	7·1	143·5	282·6
Average,	10·1	24·1	34·2	41·5	7·3	138·6	325·6

With quinine sulphate—continued.

Date. April 14.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference. c. c. oxalic acid.	CO_2 in a. b and c. mg.	CO_2 in 37·5 L. aspirated air. mg.	Body temperature. C.
	tube a. c. c.	tubes b and c. c. c.						
A. M. 8:54 to 9:24	10·0	24·3	34·3	41·5	7·2	133·4	333·5	38·6
9:50 to 10:20	10·2	24·1	34·3	41·5	7·2	133·4	333·5	38·2
10:43 to 11:13	9·8	24·0	33·8	41·5	7·7	142·6	356·7	38·3
11:38 to 12:08	10·9	24·3	35·2	41·5	6·3	116·7	291·8	37·6
P. M. 1:57 to 2:27	11·3	24·5	35·8	41·5	5·7	105·6	264·0	34·7
Average,	10·4	24·3	34·6	41.5	6·9	126·3	315·9	37·5

The following amounts of quinine were given by way of the mouth:

April 12	5:20 p. m.	0·130 gram quinine sulphate.
" 13	9:10 a. m.	0·260 " " "
" 13	1:00 p. m.	0·390 " " "
" 13	5:15 p. m.	0·520 " " "
" 14	8:45 a. m.	0·520 " " "
" 14	12:00 m.	0·780 " " "
		2·600

Rabbit died at 3 p. m. April 14.

Following are the average daily results in temperature and in the amount of carbonic acid excreted per 37·5 litres of aspirated air.

April 12	38·6 C.	317·8 milligrams CO_2.
" 13	38·1 "	325·6 " "
" 14	37·5 "	315·9 " "

SECOND SERIES OF EXPERIMENTS WITH QUININE.

Normal period, without quinine sulphate.

Date. May 17.	Oxalic acid to neutralize baryta solution. tube a. c. c.	Oxalic acid to neutralize baryta solution. tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. Ba(OH)$_2$. c. c.	Difference c. c. oxalic acid	CO$_2$ in a, b and c mg.	CO$_2$ in 37.5 L. aspirated air. mg.	Body temperature. °C.
A. M. 8:40 to 9:10	5·3	22·2	27·5	41·8	14·3	289·0	722·7	38·8
9:33 to 10:03	6·1	22·8	28·9	41·8	12·9	260·7	651·9	38·8
10:29 to 10:59	6·2	22·6	28·8	41·8	13·0	262·7	657·0	38·8
11:24 to 11:54	4·9	21·9	26·8	41·8	15·0	303·2	758·1	39·1
P. M. 1:55 to 2:25	5·0	22·1	27·1	41·8	14·7	297·1	742·9	39·0
2:48 to 3:18	4·5	22·0	26·5	41·8	15·3	309·2	773·2	38·9
3:43 to 4:13	6·7	23·1	29·8	41·8	12·0	242·5	606·5	38·8
4:35 to 5:05	6·7	23·0	29·7	41·8	12·1	244·5	611·5	38·9
Average,	5·7	22·4	28·1	41·8	13·7	276·3	690·5	38·6

May 18. *With quinine sulphate.*

Date.	tube a. c. c.	tubes b and c. c. c.	Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. Ba(OH)$_2$. c. c.	Difference c. c. oxalic acid	CO$_2$ in a, b and c mg.	CO$_2$ in 37.5 L. aspirated air. mg.	Body temperature. °C.
A. M. 9:05 to 9:35	6·2	22·5	28·7	41·8	13·1	264·8	662·0	38·6
10:00 to 11:30	7·5	23·0	30·5	41·8	11·3	228·4	571·1	38·4
10:51 to 11:21	5·6	22·1	27·7	41·8	14·1	285·0	712·6	38·3
11:46 to 12:16	6·0	22·5	28·5	41·8	13·3	268·8	672·2	38·8
P. M. 2:14 to 2:44	6·5	22·8	29·3	41·8	12·5	252·6	631·7	38·9
3:07 to 3:37	6·5	22·9	29·4	41·8	12·4	249·6	624·1	39·0
4:01 to 4:31	7·0	23·1	30·1	41·8	11·7	236·5	591·3	39·0
4:58 to 5:28	6·0	22·1	28·1	41·8	13·7	276·9	692·4	38·4
Average,	6·4	22·6	29·0	41·8	12·8	257·8	644·7	38·7

With quinine sulphate—continued.

Date. May 19.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. Ba(OH)$_2$. c. c.	Difference. c. c. oxalic acid.	CO$_2$ in a. b and c. mg.	CO$_2$ in 37·5 L. aspirated air. mg.	Body temperature. °C.
	tube a. c. c.	tubes b and c. c. c.						
A. M. 9:02 to 9:32	7·3	23·0	30·3	41·8	11·5	232·4	581·2	38·9
9:57 to 10:27	7·6	23·2	30·8	41·8	11·0	222·3	555·9	38·4
10:50 to 11:20	7·2	23·1	30·3	41·8	11·5	232·4	581·2	38·7
11:43 to 12:13	6·9	23·0	29·9	41·8	11·9	240·5	601·4	38·6
P. M. 2:03 to 2:33	7·5	23·2	30·7	41·8	11·1	223·3	558·4	38·9
2:54 to 3:24	8·2	23·6	31·8	41·8	10·0	202·1	505·4	38·5
3:47 to 4:17	7·4	23·3	30·7	41·8	11·1	224·3	561·0	38·8
4:40 to 5:10	7·1	23·1	30·2	41·8	11·6	234·5	586·3	38·8
Average,	7·4	23·2	30·6	41·8	11·2	226·4	566·4	38·7

The quinine was given by way of the mouth in gelatin capsules, in the following quantities :

May 17	5:30 p. m.	0·250 gram quinine sulphate.
" 18	8:50 a. m.	0·250 " " "
" 18	12:30 p. m.	0·250 " " "
" 18	5:35 p. m.	0·250 " " "
" 19	8:50 a. m.	0·325 " " "
" 19	1:55 p. m.	0·250 " " "
		1·575

Following are the average daily excretions of carbonic acid expressed in milligrams of CO$_2$ per 37·5 litres of aspirated air, together with the average body temperature :

May 17	38·6° C.	690·5 milligrams CO$_2$
" 18	38·7 "	644·7 " "
" 19	38·7 "	566·4 " "

THIRD SERIES OF EXPERIMENTS WITH QUININE.

Normal period, without quinine sulphate.

Time March 8.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used c. c.	Oxalic acid equivalent to 250 c c Ba(OH) c c	Difference. c c. oxalic acid	CO_2 in a. b and c mg	CO_2 in 37 5 L aspirated air mg	Body temperature °C
	tube a c c	tubes b and c c. c.						
A. M. 9:50 to 10:20	15·5	33·9	49·4	59·25	9·85	182·5	456·3	39·2
10:58 A. M. injected subcutaneously 0·083 gram quinine sulphate.								
11:15 to 11:45	16·9	34·5	51·4	59·25	7·85	145·4	363·5	38·4
12:25 to 12.55	15·3	33·9	49·2	59·25	10·05	186·2	465·5	39·9
P. M. 3:00 to 3:30	15·6	34·0	49·6	59·25	9·65	178·8	447·0	39·8
4:06 to 4:36	15·8	33·7	49·5	59·25	9·75	180·7	451·8	39·9
Average,	15·9	34·0	49·9	59·25	9·35	172 8	432·0	39·5

FOURTH SERIES OF EXPERIMENTS WITH QUININE.

Normal period, without quinine sulphate.

Time. March 10.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c c $Ba(OH)_2$. c c.	Difference. c. c. oxalic acid.	CO_2 in a, b and c, mg	CO_2 in 37 5 L. aspirated air mg.	Body temperature °C.
	tube a. c c	tubes b and c c. c.						
A. M. 9:11 to 9:41	15·0	33·8	48·8	59·25	10·45	193·6	484·0	38·8
10:15 A. M. injected subcutaneously 0·15 gram quinine sulphate.								
10:33 to 11:03	16·5	34·6	51·1	59 25	8·15	151·0	377·5	38·4
11:39 to 12:09	15·4	33·9	49·3	59·25	9 95	184·4	461 0	38·9
12:38 to 1:08	15·3	34·1	49·4	59·25	9·85	182·5	456·3	38·4
P. M. 2:54 to 3:24	16·0	34·3	50·3	59·25	8·95	165·8	414·5	39·1
4:07 to 4:37	15·9	34·1	50·0	59·25	9·25	171·4	428·5	38·8
Average,	15·8	34·2	50·0	59·25	9·25	171·0	427·6	38·7

EXPERIMENT WITH CINCHONIDINE.

Normal period, without cinchonidine sulphate.

Date. June 24.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. Ba(OH)$_2$. c. c.	Difference. c. c. oxalic acid.	CO$_2$ in a, b and c. mg.	CO$_2$ in 37·5 L aspirated air. mg.	Body temperature. ° C.
	tube a. c. c.	tubes b and c. c. c.						
A. M. 9:00 to 9:30	10·6	28·8	39·4	50·2	10·8	218·3	545·8	36·4
9:56 to 10·26	11·2	29·1	40·3	50·2	9·9	200·1	500·3	37·4
10:47 to 11:17	12·0	29·0	41·0	50·2	9·2	185·9	465·0	38·1
11:38 to 12:08	10·6	28·7	39·3	50·2	10·9	220·3	550·9	36·3
P. M. 2:03 to 2:33	10·3	28·5	38·8	50·2	11·4	230·4	576·1	36·9
2:51 to 3:21	9·4	28·2	37·6	50·2	12·6	254·9	636·8	37·1
3:42 to 4:12	9·7	28·5	38·2	50·2	12·0	242·5	606·5	37·0
4:30 to 5:00	9·7	28·5	38·2	50·2	12·0	242·5	606·5	36·4
Average,	10·5	28·6	39·1	50·2	11·1	224·3	561·0	37·0
June 25.	*With cinchonidine sulphate.*							
A. M. 9:03 to 9:33	10·8	28·6	39·4	50·2	10·8	218·3	545·8	38·6
9:58 to 10:28	11·4	28·9	40·3	50·2	9·9	200·1	500·3	38·6
10:53 to 11:23	11·1	28·7	39·8	50·2	10·4	210·2	525·6	38·6
11:48 to 12:18	11·6	28·7	40·3	50·2	9·9	200·1	500·3	38·7
P. M. 2:04 to 2:34	11·1	28·8	39·9	50·2	10·3	208·2	520·5	38·9
2:59 to 3:29	11·5	29·0	40·5	50·2	9·7	196·0	490·2	39·0
3:56 to 4:26	11·6	29·0	40·6	50·2	9·6	194·0	485·2	39·0
4:50 to 5:20	12·1	29·1	41·2	50·2	9·0	181·9	454·8	39·1
Average,	11·4	28·8	40·2	50·2	10·0	201·1	502·8	38·8

Following are the average daily amounts of carbonic acid excreted, expressed in milligrams per 37·5 litres of aspirated air, together with the average body temperature.

June 24,	37·0° C.,	561·0 milligrams CO$_2$.
" 25,	38·8	502·8 " "

Following are the doses of cinchonidine :

June 24,	5:12 P. M.,	1·000 gram cinchonidine sulphate.
" 25,	10:35 A. M.,	0·250 " " "
" 25,	12:35 P. M.,	0·325 " " "

In the second series of experiments, smaller doses of quinine were employed, so that no special toxic action was observed. In this case there seemed to be a gradual falling off in the amount of carbonic acid produced; such a decrease as might be assumed would naturally result from diminished proteid metabolism.

The results are shown in the accompanying tables.

The body temperatuie, as determined per rectum did not show any change whatever under the influence of this quantity of quinine (total dose 1·575 gram of quinine sulphate). There does not appear to be any proof that moderate doses of quinine lower the body temperature of healthy animals or even man, and Kerner found in his experiments that a full dose of quinine given to a healthy man would prevent the usual rise of temperature resulting from vigorous exercise, but did not affect the temperature under ordinary circumstances. Liebermeister* has also reported that the alkaloid has no constant depressing action on the bodily heat in health.

Two other short series of experiments were tried with quinine, also with rabbits in a condition of hunger. In both of these cases the quinine was introduced by sub-cutaneous injection in the form of sulphate. In both cases there was a slight fall in temperature, accompanied with a noticeable decrease in the amount of carbonic acid eliminated, directly after injection of the quinine. This effect, however, was only temporary, for the temperature quickly rose to the normal, and even somewhat above the normal point, while the carbonic acid in the third series came quite back to the normal and in the fourth series remained only a little way below.

It would appear, therefore, from our experiments, that in a healthy, hungry rabbit moderate doses of quinine sulphate exercise at the most only a very slight depressing influence on body temperature, and have but a minimum effect on the production of carbonic acid.

Action of cinchonidine sulphate.

Previous experiments† on man have shown that cinchonidine has the power of lessening materially the elimination of nitrogen, presumably through its inhibitory action on proteid metabolism.

Cinchonidine is supposed to have much the same physiological action as quinine and cinchonine, only weaker. Our present experiments with the alkaloid, using quite large amounts of the sulphate, show a somewhat different action from quinine. Using a large rabbit, without food for three days, and giving it by way of the mouth, in gelatin capsules, large doses of the alkaloid, there was a very noticeable and constant rise in temperature up to the very time of death, accompanied by a slight but gradual diminution in the amount of carbonic acid given off. In all, 1·575 grams of cinchonidine sulphate were given; an amount exactly equal to the quinine sulphate given in the second series of experiments with quinine. With cinchonidine, however, the rabbit was much prostrated, showed symptoms of tetanic convulsions, and finally died in a vigorous tetanic spasm at the end of the second day.

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* Deutsch. Archiv fur Klinische Medicin, Band iii.

† See Chittenden and Whitehouse, Studies from the Laboratory of Physiological Chemistry, vol. i. p. 164.

The results are shown in the preceding table.

Action of Antipyrine.

Antipyrine or dimethyloxychinicine has of late been much experimented with. Among the many statements which we have seen recently concerning its action are the following, which are of interest in this connection. Arduin[*] found that 3 grams given to a rabbit produced cataleptic stiffness, diminished reflexes, etc., followed by violent convulsions. There was also a very marked fall of bodily temperature. Anscroff,[†] by experiments on animals, found that the alkaloid caused an increase of blood pressure and a decrease of internal temperature, as shown by a thermometer in the rectum, but a considerable rise in the external temperature, sometimes as much as 12° C. Pavlinoff[‡] has reported that antipyrine produces a very considerable quickening of the respiration, while Dr. Walter, of St. Petersburg,[§] is reported as having found that the alkaloid while reducing febrile temperature, also reduces nitrogenous tissue changes; and further, that the assimilation of proteids is materially favored by the drug. F. Müller[‖] has also found that in fever antipyrine diminishes the excretion of nitrogen. Coppola,[¶] however, states that in the case of a dog, 0·3–0·4 gram of antipyrine was wholly without action on its excretion of nitrogen. Coppola has further found that the

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[*] Abstract in Therapeutic Gazette, 3d series, vol i, p. 677.

[†] Abstract in Therapeutic Gazette, 3d series, vol. ii, p. 315.

[‡] Abstract in Therapeutic Gazette, 3d series, vol ii, p. 339.

[§] Abstract in Therapeutic Gazette. 3d series, vol. ii, p. 53.

[‖] Jahresbericht für Thierchemie, xiv, 242.

[¶] Jahresbericht fur Thierchemie. xv, 97.

FIRST SERIES OF EXPERIMENTS WITH ANTIPYRINE.

Normal period, without antipyrine.

Date.	Oxalic acid to neutralize baryta solution		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c c $Ba(OH)_2$. c. c.	Difference c c. oxalic acid.	CO_2 in a, b and c mg.	CO_2 in 37·5 L. aspirated air. mg	Body temperature. °C.
May 24	tube a. c. c.	tubes b and c. c c.						
A. M. 8:54 to 9:24	8·6	26·4	35·0	46·1	11·1	224·3	561·0	38·4
9:52 to 10:22	8·8	26·5	35·3	46·1	10·8	218·3	545·8	38·4
10:47 to 11:17	9·5	26·4	35·9	46·1	10·2	205·1	513·0	38·4
P. M. 1:55 to 2:25	9·1	26·3	35·4	46·1	10·7	216·3	540·8	38·3
2:47 to 3:17	9·5	26·4	35·9	46·1	10·2	205·1	513·0	38·6
3:40 to 4:10	9·1	26·5	35·6	46·1	10·5	212·2	530·6	38·4
4:35 to 5:05	8·8	26·3	35·1	46·1	11·0	222·3	555·9	38·4
Average,	9·0	26·4	35·4	46·1	10·7	214·8	535·7	38·4
May 25.	*With antipyrine.*							
A. M. 8:56 to 9:26	9·6	26·4	36·0	46·1	10·1	204·1	510·4	38·2
9:53 to 10:23	9·6	26·4	36·0	46·1	10·1	204·1	510·4	38·1
10:49 to 11:19	7·5	25·6	33·1	46·1	13·0	262·7	657·0	38·0
11:47 to 12:17	8·4	25·9	34·3	46·1	11·8	238·5	596·3	38·1
P. M. 2:15 to 2:45	7·9	26·0	33·9	46·1	12·2	246·6	616·6	38·1
3:15 to 3:45	8·7	25·8	34·5	46·1	11·6	234·5	586·3	38·3
4:15 to 4:45	8·4	26·0	34·4	46·1	11·7	236·5	591·3	38·0
5:09 to 5:39	7·4	25·6	33·0	46·1	13·1	264·8	662·0	38·1
Average,	8·5	25·9	34·4	46·1	11·7	236·5	591·3	38·1
May 26.								
A. M. 9:02 to 9:32	11·3	26·8	38·1	46·1	8·0	161·7	404·3	36·2
9:59 to 10:29	9·4	26·0	35·4	46·1	10·7	216·3	540·8	35·3

alkaloid not only has a noticeable antipyretic action in conditions of fever, but also reduces the temperature in healthy organisms. The extent of reduction, however, is not great, ranging only from 0·1 to 0·6 of a degree. Further, the diminution in temperature is to be ascribed, according to Coppola, not to diminished metabolic activity, but to increased giving up of heat, due to dilatation of the blood-vessels by the antipyrine. Jacubowitsch* also claims for antipyrine an inhibitory action on the excretion of uric acid. So far as our knowledge extends, however, no experiments have been tried as to the influence of this therapeutic agent on the production of carbonic acid.

Our experiments have been confined wholly to rabbits, and those in a condition of hunger. In the first series of experiments, during the antipyrine period, the alkaloid was given in large and oft-repeated doses, in the form of powder, in gelatin capsules, as follows:

May 25,	8:33 A. M.,	0·2 gram antipyrine.
“ 25,	9:35 “	0·2 “ “
“ 25,	10:30 “	0·2 “ “
“ 25,	11:27 “	0·2 “ “
“ 25,	12:27 P. M.,	0·6 “ “
“ 25,	2:55 “	0·6 “ “
“ 25,	3:54 “	0·6 “ “
“ 25,	4:51 “	0·6 “ “
“ 25,	5:46 “	0·6 “ “
“ 26,	8:45 A. M ,	0·6 “ “
“ 26,	9:38 “	1·0 “ “
		5·4

The accompanying tables show the results obtained. At the end of the last period of the second day (May 26) respiration was very rapid, about 208 per minute, and during that period the amount of carbonic acid excreted was larger than in any other. In spite of the large dose taken, however, the alkaloid appears to have had no special action on the production of carbonic acid, and further, the temperature was only very slightly lowered until on the last day, just prior to the animal's death. On the last day of the experiment (May 26) the rabbit appeared much prostrated, and at 10.35 A. M. was seized with a convulsion, followed soon after by three others, dying at 11.30 A. M.

In the second series of experiments somewhat smaller amounts of antipyrine were given, but here, as in the first series, there was no

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* Jahresbericht fur Thierchemie, xv, p. 444.

SECOND SERIES OF EXPERIMENTS WITH ANTIPYRINE.

Normal period, without antipyrine.

Date. May 31.	Oxalic acid to neutralize baryta solution		Total oxalic acid used. c. c.	Oxalic acid equivalent to 250 c. c. $Ba(OH)_2$. c. c.	Difference c. c oxalic acid.	CO_2 in a, b and c. mg	CO_2 in 37·5 L. aspirated air. mg	Body temperature °C.
	tube a c. c	tubes b and c. c c						
A. M. 9:02 to 9:32	8·5	25·3	33·8	44.3	10·5	211·2	528·1	38·9
9:57 to 10:27	8·1	25·1	33·2	44·3	11·1	224·3	561·0	38·9
10:59 to 11:29	9·1	25·5	34·6	44·3	9 7	196·0	490·2	38·9
11:51 to 12:21	9·3	25·5	34·8	44·3	9·5	191·0	477·6	38·9
P. M. 1:44 to 2:14	9·8	25·7	35·5	44·3	8·8	176·8	442·2	38·7
·3:30 to 4:00	9·3	25·5	34·8	44·3	9·5	192·0	480·1	38·9
4:21 to 4:51	9·7	25·7	35·4	44·3	8·9	179·9	449·8	38·8
Average,	9·1	25·5	34·6	44·3	9·7	195·9	489·9	38·8

June 1. *With antipyrine.*

A. M. 8:53 to 9:23	9·9	25·5	35·4	44·3	8·9	179·9	449·8	38·7
9:50 to 10:20	9·4	25·5	34·9	44·3	9·4	190·0	475·1	38·9
10:43 to 11:13	10·1	25·7	35·8	44·3	8·5	170·8	427·0	38 9
11:40 to 12:10	9·7	25 7	35·4	44·3	8·9	179·9	449·8	38·7
P. M. 2:15 to 2:45	9·6	25·6	35·2	44·3	9·1	183·9	459·9	39·2
3:12 to 3:42	9·9	25·8	35·7	44·3	8·6	173·8	434·6	38·4
4:11 to 4:41	9·7	25·6	35·3	44·3	9·0	181·9	454·8	38·3
5:05 to 5:35	8·7	25·3	34·0	44·3	10·3	208·2	520·5	38·4
Average,	9·6	25·6	35·2	44·3	9.08	188·5	458·9	38·7

With antipyrine—continued.

Date. June 2.	Oxalic acid to neutralize baryta solution.		Total oxalic acid used c. c.	Oxalic acid equivalent to 250 c. c. Ba(OH)$_2$. c. c.	Difference. c c oxalic acid.	CO$_2$ in a. b and c. mg.	CO$_2$ in 37·5 L. aspirated air. mg.	Body temperature. °C.
	tube a c. c.	tubes b and c. c. c.						
A. M.								
8:56 to 9:26	10·0	25·5	35·5	44·3	8·8	177·8	444·7	38·7
9:53 to 10:23	9·8	25·6	35·4	44·3	8·9	179·9	449·8	38·6
10:47 to 11:17	8·8	25·3	34·1	44·3	10·2	206·1	515·5	37·8
11:43 to 12:13	8·6	25·1	33·7	44·3	10·6	214·2	535·7	37·1
P. M.								
2:02 to 2:32	8·6	25·1	33·7	44·3	10·6	213·2	533·2	37·1
2:55 to 3:25	8·7	25·1	33·8	44·3	10·5	212·2	530·6	37·3
3:54 to 4:24	8·8	25·3	34·1	44·3	10·2	206·1	515·5	37·6
4:47 to 5:17	8·5	25·1	33·6	44·3	10·7	216·3	540·8	37·5
Average,	8·9	25·3	34·2	44·3	10·06	208·2	508·2	37·7

Following are the average daily excretions of carbonic acid, expressed in milligrams per 37·5 litres of aspirated air, together with average daily body temperature:

May 31	38·8° C.	489·9 milligrams CO$_2$.
June 1	38·7	458·9 " "
" 2	37·7	508·2 " "

Antipyrine was given in the following quantities by mouth, in gelatin capsules:

May 31	4.57 p. m.	0·2 gram antipyrine.
June 1	8:40 a. m.	0·2 "
" 1	9:31 "	0·2 "
" 1	10:25 "	0·2 "
" 1	11:20 "	0·2 "
" 1	12:17 p. m.	0·2 "
" 1	2:52 "	0·2 "
" 1	3:49 "	0·2 "
" 1	4:48 "	0·2 "
" 1	5:40 "	0·2 "
" 2	8:45 a. m.	0·5 "
" 2	9:34 "	0·5 "
" 2	10:31 "	0·5 "
" 2	11:24 "	0·5 "
" 2	4:45 p. m.	0·2 "
		4·2

discernible action on the excretion of carbonic acid. Further, the alkaloid did not noticeably lower the body temperature until toward the close of the second day, when the quantity given had reached an amount nearly sufficient to produce a fatal result. We have then to conclude that antipyrine, at least in therapeutic doses, has no special influence on the production or elimination of carbonic acid by the rabbit.

XXIV.—NEW ENGLAND SPIDERS OF THE FAMILY CINIFLONIDÆ.

BY J. H. EMERTON.

THE spinning organs of the Ciniflonidæ differ from those of all other spiders. They have in front of the usual spinnerets an additional spinning organ, the *cribellum*, with spinning tubes like the other spinnerets, but much finer, and they have on the metatarsus of each hind leg a row of stiff hairs, the *calamistrum*, by which the thread is combed from the cribellum in a loose curly band. This band of loose thread forms part of every cobweb made by these spiders (Pl. x, fig. 1*g*.) and is easily distinguished in new webs by its width and white color and in old webs by the amount of dust which it collects.

The feet have three claws and some species have a few curved and toothed spines under the claws, like *Epeiridæ* and *Theridiidæ*. The tracheæ are large and open in a wide slit in front of the cribellum.

The colors are generally dull brown and grey. A double row of oblique light markings on the back of the abdomen, which shows most distinctly in *Amaurobius*, is in a modified from the usual marking of the abdomen throughout the family, often varying greatly in shape in the same species.

These spiders were first placed together in one family by Blackwall, who in 1839 noticed their peculiar webs and spinning organs. Before that time they had been scattered among various families according to their size, form, and habits. They have been treated in the same way by Thorell in his book on the genera of European spiders, and by Menge in the spiders of Prussia. Simon divides the French species into two families, *Dictynidæ* and *Uloboridæ*. Bertkau in his latest revision of the family, in 1882, carries the division into families still further and unites them all into a sub-order, *Cribellata*.

I have followed Blackwall in considering the group as one family, and use his name *Ciniflonidæ*. The sub-family *Uloborinæ* of Thorell

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J. Blackwall. On the number and structure of the mammulæ employed by spiders in the process of spinning. Trans. Linn. Soc. London, vol. xviii, 1839.

P. Bertkau. Cribellum and Calamistrum. Archiv fur Naturgeschichte, 1882.

I use in the same sense, transferring it from the *Epeiridæ* to this family.

Several of the spiders described by Hentz under the name of *Theridion* are probably *Dictyna*. Of these, *T. sublatum*, *morologum*, and *foliaceum* belong to this genus without much doubt, though I cannot identify them with any species here described.

Blackwall mentions among spiders from Canada, *Ergatis* (*Dictyna*) *diligens*, *var. annulipes*, Ann. and Mag. of Nat. Hist., 1871.

B. G. Wilder describes the webs and habits of *Hyptiotes cavatus* under the name *H. Americanus*, in Popular Science Monthly, 1875.

E. Keyserling has described in Transactions of the Zool. Botan. Gesellschaft of Vienna, 1881 to 1884, the following species: *Dictyna sedentaria*, Baltimore, Coll. of L. Koch. *D. volupis*, Museum, Cambridge, Mass. *D. volucripes*, Museum, Cambridge, Mass. *D. foliata*, Colorado, Vienna Museum. *D. vittata*, Washington, D. C., Warsaw Museum. *D. arundinaceoides*, Cañon City, Colorado, Coll. of G. Marx.

Dictyna Sundevall.

The genus *Dictyna* is composed of small spiders that live in loose webs of various shapes on fences and on plants, especially on the ends of stalks and among the flowers of *Solidago*, *Spiræa*, and other slender plants with clusters of small flowers.

The head is generally high, but not so wide as in *Amaurobius*. The sternum is very wide and convex and the labium large, often nearly as long as the mandibles. The tracheæ are large and the opening generally distinct. The difference between the sexes in most species is very great. The male palpi are very large and the palpal organs conspicuous. The tibial joint of the male palpi has on the outer side a process with two short spines. The mandibles of the males are bowed outward (Plate IX, fig. 2d) and are much longer than those of the female. They are bent forward at the tips, and at the base of each mandible is a short tooth projecting forward. Plate IX, fig. 2*b*.

The colors are usually dull yellow and brown covered with white or gray hairs. The cephalothorax is usually lighter in front and dark at the side, and the abdomen has a double row of light markings in the middle on a dark ground, but these markings are extremely variable even in the same species. Pl. IX.

The webs of *Dictyna* usually radiate irregularly from a hole or hiding place where the spider hangs. Some species, living on walls, make a round patch of web with the hole near the center, but usually the shape of the web depends on that of the plant on which it is made. The principal threads of the web, if they are parallel or radiating slightly, are often crossed by a number of parallel short threads, like a segment of a web of *Epeira* (Pl. XI, fig. 3) and the curled band is carried back and forth on these as in the figure of the web of *Amaurobius*. Pl. X, 1*g*.

Dictyna muraria, new sp.

PL. IX, FIGURES 1 TO 1*g*.

Length about 3^{mm}. The cephalothorax is dark brown, a little lighter on the top of the head with a few gray hairs in longitudinal rows. The abdomen resembles that of *D. volucripes* Keys, but the middle dark markings are wider in front and more broken behind. In the middle of the front half is a wide dark patch, extending about to the middle of the abdomen. Behind this are two rows of dark spots connected by transverse lines, more or less complete, forming an Epeira-like marking. Pl. IX, figs. 1 to 1*e*.

The markings of this species and of *volucripes* vary greatly, so that they often cannot be distinguished by them. The metatarsus of the hind legs is nearly straight, not so much curved as in *volucripes*.

The males are darker, but usually have the same markings. Their abdomen is smaller than that of the females, but the cephalothorax is fully as large. The male palpi resemble those of *volucripes* Keys. The tibia is similar in shape, but is proportionally longer, and the two-spined process shorter than in *volucripes*. Pl. IX, 1*f*, 1*g*.

This spider is found all over New England. It is the most common species on fences, but is found also on plants, and, in winter, under leaves. It sometimes tries to fly, oftener in the spring than in the fall, which is the usual flying time of most spiders. I have specimens from Mt. Washington, N. H.; Portland, Me.; Salem, Mass.; Albany, N. Y.; New Haven, Conn.; Wood's Holl, Mass.; and several places around Boston.

A female in the Museum of Zoölogy, Cambridge, Mass., named by Keyserling, *D. arundinaceoides* Keys., is perhaps this species. It has the abdomen very much distended, so that the epigynum shows much plainer than usual. The spider first described by Keyserling as *D. arundinaceoides* came from Colorado, and I have not seen it and do not feel sure enough of its identity to adopt the name for this species.

Dictyna volucripes Keyserling, Zool Botan Gesellschaft, Vienna, 1882

Pl IX, FIGURES 2 TO 2f AND Pl XI, FIGURE 3

Female, 3·5mm long or longer. The male is nearly as large, but the cephalothorax is larger and the abdomen smaller.

The cephalothorax is dark reddish brown, and partly covered with white or gray hairs, most of them arranged in several lines from the dorsal groove to the eyes.

The abdomen has an irregular dark figure in the middle, narrow in front and widening backward. On each side of this is a light gray area, which becomes yellow in alcohol, and below these the sides and under surface of the abdomen are dark brown with some light markings. The abdomen is covered with gray hairs which modify the color. The legs are brown, usually lighter than the thorax, and covered with gray hairs.

The male is a little darker than the female. The male palpi are short, and large at the end. The patella is as wide as long. The tibia is a little longer than the patella and widened on the outer side at the distal end, so as to be as wide there as long. The two-spined process is as long as the tibia is wide, and is on the upper side of the tibia. The tarsus and palpal organ are large. Pl. IX, figs. 2*e*, 2*f*. The two spined process varies in form. It is usually curved forward, but in some specimens is nearly straight.

This species lives most commonly in thick and irregular webs on the ends of plants. The dried tops of *Spiræa* and *Solidago* are favorite places for it. It also lives occasionally on fences. All over New England.

Dictyna longispina, new sp

PL. IX, FIGURE 4.

This species resembles *volucripes*, but is a little smaller. The markings of the abdomen are similar, but the cephalothorax and legs are lighter and redder.

The plainest difference of this species from the others is in the shape of the tibia of the male palpus. This is very long, as long as the femur, and stouter. The two-spined process is as long as the tibia, and extends backward nearly parallel to it. Pl. IX, fig. 4. The palpal organ extends farther backward than usual. The end of the tube and the accompanying process extending in a spiral nearly to the base of the tibia.

A young female, similarly marked and colored, accompanies the male and is probably the same species.

Meriden, Conn., one male and one female.

Dictyna bostoniensis, new sp

PL. IX, FIGURES 3 TO 3*d*

This is a rather large species, measuring 4^{mm} or more in length, but the cephalothorax is small and the abdomen much larger than in most species. The legs are whitish. The cephalothorax of the female is the same color in the middle, but darker and streaked with radiating brown lines on the sides. The abdomen is white with gray or black markings. Pl. IX, figs. 3*b*, 3*c*, 3*d*. In the middle of the front half of the abdomen is an irregular dark stripe extending over the first and second segments. Behind this are two rows of irregular spots, about one-third the width of the abdomen apart. The sides are marked by a few dark spots in broken oblique lines. The sternum and under side of the abdomen are white, with a few irregular dark spots. The spider resembles a piece of bird dung.

The cephalothorax of the male is larger and darker colored. The male palpi are short and slender. The tibia is short and as wide at the distal end as it is long. The two-spined process is short and on the outer side. The tarsus and palpal organ are small. Pl. IX, fig. 3*a*.

In 1873, this spider lived in great numbers on the iron fence around the public garden in Boston, making webs in corners, with an open tube in which the spider stood. Single specimens were found in Beverly and Brookline. In 1886, it was rare on the public garden, but common on the fences of the Back Bay park on Beacon street. I have not found it in other parts of New England.

Dictyna minuta, new sp.

PL. IX, FIGURES 5, 5*a*.

About 2^{mm} long. The markings are like those of *D. muraria*, but the colors are lighter and redder than in that species, and the only two specimens are much smaller.

The legs are very light brownish yellow, darker toward the base. The sternum and labium are reddish brown, and both are large and wide in proportion to the size of the spider.

The tibia of the male palpus is about twice as long as the patella, and nearly straight. The two-spined process is short and turned slightly forward. The spines are large and black. The point of the palpal organ is long and slender and twisted loosely. In both specimens it reaches backward half the length of the tibia. Pl. IX, figs. 5, 5*a*.

Two specimens only, from Hamden, Conn., and Providence, R. I.

Dictyna rubra, new sp

Pl. IX, figure 7.

Female 2·5^{mm} long. The color is more red than in the other species. The cephalothorax and legs are light orange-brown. The abdomen is darker reddish brown, with several yellow cross lines on the hinder half, and in some individuals a yellow patch on the front half. The sternum, maxillæ, and mandibles are light, like the legs.

The male palpi are moderately large. The tibia is a little longer than wide, and as thick at the base as at the tip. The two-spined process is on the upper side of the tibia, close to the base. It is as long as the tibia is thick. The tarsus is small. Pl. ix, fig. 7.

The abdomen is more pointed behind than in most species.

It lives on plants, but I do not know its web. Common in eastern Massachusetts and around New Haven, Conn.

Dictyna cruciata, new sp.

Pl. IX, figures 6, 6*a*.

This is the lightest colored species. The cephalothorax of the female is yellowish white in the middle and light brown at the sides. In the male the whole cephalothorax is dull yellow.

The abdomen is white in the middle, the color spreading down the middle of each side, forming in many individuals a cross-shaped marking (Pl. ix, fig. 6). The sides are light brown. The legs are yellowish white.

The male palpi are large and the palpal organs wide. The tibia is short, not much longer than wide, and the two spines are short and on a low process of the tibia. Pl. ix, fig. 6*a*.

Eastern Mass.; New Haven, Conn.

Dictyna volupis Keyserling, Zool. Botan. Gesell., Vienna, 1882.

Pl. IX, figures 8 to 8*c*.

This is one of our most common spiders throughout the summer. It lives under leaves and between the twigs of trees and shrubs of all kinds, making small and thin webs.

The female is about 3^{mm} long. The legs and front part of the cephalothorax are yellowish white. The sides of the cephalothorax are brown. The abdomen has usually an irregular light yellow marking in the middle and is light brown or reddish at the sides. Pl. ix, fig. 8. Some individuals are without the yellowish marking on the back and have the abdomen brownish all over, covered with whitish hairs. The reddish markings all become redder in alcohol. The sternum and under side of the abdomen are light yellow.

The colors of the male are very different. The whole cephalothorax is orange-brown, not much darker at the sides. The abdomen is reddish brown, darker than in the female, with only a little yellow in the middle, and sometimes none. The legs are darker yellow than in the female.

The males and females are about the same size, but differ in form as much as they do in color. The front of the head is low in both sexes, and rises backward to its highest point midway between the eyes and the dorsal groove. In the males the mandibles are so long that the distance from the top of the head to the end of the mandibles is as great as the length of the cephalothorax. The male mandibles are concave in front and bowed widely apart in the middle. Even the females have the mandibles a little concave in front.

The male palpi are long and large. The tibia is twice as long as wide; thickened at the end, and curved downward. The two-spined process is short and a little in front of the base of the tibia. Pl. IX, fig. 8*c*.

The palpal organ is unusually large, and the long tube can be seen passing around it under the edges of a large thin appendage. Pl. IX, fig. 8*a*, 8*b*.

The webs are spread under leaves or between twigs.

I have twice seen the pairing of this species. In one case the female stood across a forked twig and the male reached up from below, his head being under hers and his mandibles parallel to her sternum. In the other the male and female stood head to head in the web, the cephalothorax of the female being tipped up in front, and resting upon the head and mandibles of the male.

Common all over New England. In winter they are often found under leaves, half grown, and soon get to their growth when warm weather begins. Several small, flat egg-cocoons are fastened under a leaf and there may be several broods in each season.

Dictyna frondea, new sp.

PL. IX, FIGURES 9, 9*a*

This species is a little smaller than *volupis* and is similarly colored in the female, except that it is usually a little darker and less red. The cephalothorax is light brown, a little lighter in the middle of the head, but not so much so as *volupis*. The abdomen is brown at the sides and yellow in the middle. The yellow stripe is narrower and straighter than in *volupis*, and often forms a regular herringbone figure. Pl. IX, fig. 9. The under side of the abdomen is nearly as dark as the upper side, and the sternum has the same color while *volupis* is usually light colored beneath.

The males differ less from the females than in *volupis*. They are colored a little darker than the females. The light stripe on the abdomen is narrower and in some individuals wanting. The male palpi are long and slender. The tibia is more than twice as long as wide. The two spined process is very small and close to the base of the tibia. The tarsus is smaller than in *volupis*, and the palpal organ very much smaller and more simple. Pl. IX, fig. 9*a*.

On grass and low bushes all over New England.

This species (or *D. volupis*) is probably Hentz's *Theridion foliaceum*.

Amaurobius C. Koch

The genus *Amaurobius* contains our largest spiders of this family. In general appearance they resemble the stouter species of the genus *Tegenaria*, but do not have the long upper spinnerets of that genus. The head is large and high, and wide in front. The eyes are in two rows, only slightly curved, and are all small and of nearly the same size.

The maxillæ are long, and at the tip curve inward a little over the labium. The labium is longer than wide and a little narrowed at the tip. The mandibles are very large and strong.

The whole body is thickly covered with fine, short hair, giving it a soft velvet-like appearance. The spines on the legs are small and concealed by the hair.

The calamistrum consists of two rows of hairs, those of the outer row being much curved and close together, and those of the inner row three or four times as far apart. Pl. x, fig. 1*f*. The cribellum is long and narrow and divided in the middle. Pl. x, fig. 1*e*.

The colors of all our species are much alike. The cephalothorax and legs are dark brown, except in freshly moulted or young specimens, and the abdomen is dark gray with a double row of oblique light markings.

The webs are large and loose, often filling a cavity in a rotten log or under stones. There appears to be little regularity in the shape of the web or arrangement of the threads. The whole web is made of smooth silk and the band of curled threads is afterwards attached to parts of it as in Pl. x, fig. 1*g*.

The sexes are about equal in size. The male palpi are large. Their tibial joints are short and wide and furnished with large processes of various shapes.

Amaurobius sylvestris, new sp.

Pl. X, figures 1 to 1*g*.

This is the common *Amaurobius* all over New England. The female is 10^{mm} long with the cephalothorax 5^{mm} long. The head is nearly as wide as the thorax. It is low in front and rises to its highest point half way to the dorsal groove. The cephalothorax is dark brown, darkest on the front of the head. The legs are dark brown, usually lighter than the thorax. In the young the colors are all much paler.

The abdomen is oval, widest behind. It is dark greenish gray with a double row of oblique yellow or white markings on the hinder half, and two curved markings of the same color on the front. These markings run together, forming a figure which varies greatly in form and size in different individuals. Pl. x, fig. 1.

The males differ but little from the females. The male palpi are large. The tibia is short and wide and has three long processes, the inner of which is slender and pointed and nearly twice as long as the tibia (Pl. x, figs. 1*a*, 1*b*.) but not so much curved as in figures of the European *A. claustrarius*.

The epigynum is small, the middle lobe is small and the side lobes meet behind so as to completely surround it (Pl. x, fig. 1*e*) which is very different from the epigynum of *A. claustrarius* as figured by Koch.

This species lives under stones, under leaves, and in the hollows of rotton trees and stumps. Fig. 1*g* is part of a web, showing the arrangement of the curled threads.

All over New England. In the White Mountains up to the highest trees.

Three specimens of this species in the museum of Comp. Zoölogy, Cambridge, are named by Keyserling *A. claustrarius*, which this species closely resembles. I have only young *claustrarius* for comparison, but judging by descriptions and figures, especially those of L. Koch in Abh. Nat. Gesellsch. of Nuremburg, 1868, I do not believe them the same species.

Amaurobius ferox (Walck.) Koch., *Ciniflo ferox* Blk.

Pl. X, figures 3 to 3*c*.

This is our largest species. It is found only about houses and cellars, and is probably imported, as it is a common spider in Europe.

The female is 12^{mm} long. Cephalothorax 6^{mm} long, 4^{mm} wide. The head is 3^{mm} wide and highest half way between the eyes and the dorsal groove.

The cephalothorax is yellowish brown, darkest in front and nearly black around the eyes. The legs are the same color as the thorax, darkest toward the tips. The abdomen is dark gray with light yellowish marks on the back. On the front half of the abdomen are a middle and two lateral stripes and behind these four or five pairs of oblique markings.

The eyes are all small and about equal in size. The front row is about half the width of the head and the eyes equidistant. The upper row is longer and the lateral eyes considerably farther from the middle ones than these are from each other. The mandibles are large and strong.

The calamistrum is a double row of spines, half the length of the hind metatarsus.

The male differs but little from the female, except that the abdomen is a little smaller and the front legs longer. The male palpi are very large. The tibia is as short as wide. It is bent inward, and has a large spine on the outer and another on the upper side, each nearly as long as the tibia. On the inner side is a third smaller spine. Pl. x, figs. 3*a*, 3*b*, 3*c*. The tarsus and palpal organ are large and round. Fig. 3*a*. The epigynum is large and dark colored. The middle lobe is large and enclosed by the others only at the sides. Pl. x, fig. 3.

Boston, Salem, Beverly, Mass.; Providence, R. I.; Albany, N. Y.; New Haven, Conn., in cellars and houses.

Amaurobius tibialis, new sp.

PL. X, FIGURES 3 TO 3*c*.

Female 8^{mm} long.

The cephalothorax is light brownish yellow, not darker in front. The legs are of the same color and not much darker at the tips.

The light markings on the abdomen are united into a middle band with oblique branches at the sides on the hinder half.

The middle lobe of the epigynum is entirely concealed, the lateral lobes divided by a groove in the middle. Pl. x, fig. 2.

The middle process on the tibia of the male palpus is short, but the other processes are much larger than in the other species. The outer one is about as long as the tibia is wide, and has a large hook on the inner side. The inner process is long and slender, curving over the back of the tarsus and extending nearly to the end of it. Pl. x, figs. 2*a*, 2*b*.

This species is found on Mt. Washington, N H., up to the highest trees.

Titanœca Thorell.

Titanœca americana, new sp.

PL. X, FIGURES 4 TO 4*d*.

This spider resembles *T. quadriguttata* of Europe, but is usually without markings on the abdomen. The female is 5 or 6^{mm} long, resembling in size and shape the common *Steatoda borealis*, from which, however, it is readily distinguished by its black color.

The cephalothorax is dull orange-color, blackish around the edges and toward the front. The rest of the body is deep black and covered with long hair, except in some individuals a few light gray spots in pairs on the abdomen.

The sternum is as wide between the second legs as it is long. The labium is as wide as long, a little narrowed and rounded at the tip. The maxillæ are nearly straight on the inner edges not curved inward at the tips, as in *Amaurobius*. The head is not so wide as in *Amaurobius*. The eyes have nearly the same arrangement, but are proportionally larger. Pl. x, fig. 4. The spinnerets are short.

The cribellum is divided in the middle as in *Amaurobius*, and the calamistrum is like that genus.

The claws of the feet are large and strong, proportionally larger than those of *Amaurobius*.

Like most of the genus, this lives under stones in the driest and hottest places. Under the loose stones under the trap hills around New Haven and Meriden, Conn. it is common. I have a few from Mt. Monadnock, N. H., but have not found it elsewhere in New England.

Titanœca brunnea, new sp.

PL. X, FIGURES 5 TO 5*c*.

This species is about as large as *T. americana*, but is a little more slender and less hairy. The cephalothorax is light or dark brown, like dead oak leaves among which it lives. The joints of the legs are darker toward the distal ends. The abdomen is similarly colored, but becomes redder than the rest of the body in alcohol. Across the back are four or five lines of light yellowish spots, and there are larger irregular spots along the sides, as in many species of *Dictyna*. Pl. x, figs. 5, 5*a*. Under the abdomen between the spinnerets and epigynum are two large light spots. Fig. 5*a*.

Besides the color, the only plain difference between this and the black species is in the palpi of the female, which in this species have the last two joints a little stouter than in *T. americana*. In both species the palpi are very spiny at the end, though the spines are concealed by hairs. I have found this species three times under leaves in woods near New Haven, Conn.

Uloborinæ Thorell.

These spiders have been classed by most authors among the *Epeiridæ* on account of their resemblance to *Tetragnatha*, and especially on account of their round, or at least radiate, webs. The arrangement of the eyes, the mouth parts, and the tracheæ are all different from the *Epeiridæ*. The spinning organs include the cribellum and calamistrum, like the other *Ciniflonidæ*, and the cross-threads of the webs are partly made of curly threads spun by the calamistrum and not covered with a liquid in drops like the webs of *Epeiridæ*.

The adhesive thread of these spiders is not made separately and attached to old threads as it is by *Amaurobius* and *Dictyna*, but both threads are spun at the same time. Pl. xi, figs. 2*i*, 2*j* show both sides of a piece of the cross-threads of the web of *Hyptiotes*.

Uloborus Latreille. *Veleda*, Blackwall. *Phillyra*, Hentz.

Uloborus plumipes Lucas = *Phillyra ripaira*, Hentz.

Pl. XI, figures 1 to 1*f*.

The female is about 5mm long. Cephalothorax 1·5mm. The cephalothorax is flat in front, and extends forward in the middle beyond the mandibles. Behind it is wide and swelled up on each side, where the abdomen hangs over it. Pl. xi, fig. 1*b*.

The abdomen is narrow and slightly notched in front and extends over the cephalothorax a quarter of its length. The abdomen is widest and highest a third of its length from the front, and at this point has a pair of humps.

The colors are very variable. A dark, plainly marked female has the femur and patella of the front legs dark brown or nearly black, and the tibia dark brown, except a white ring at the base; at the end of the tibia is a brush of coarse, dark brown hairs. The tarsus and metatarsus are white. In lighter individuals the color of the femur or tibia may be broken by a white ring near the middle. The other legs have femur, tibia and metatarsus dark brown, divided near the middle by a white ring. Patella and tarsus brown, lighter at the ends. The cephalothorax is dark brown with a narrow, indistinct light line in the middle. In lighter individuals this stripe is wider.

The dorsal markings of the abdomen are more variable and less definite. On the front of the abdomen are two light spots, behind which are two very dark ones, sometimes united into one. Behind these are two white spots half as far apart as the humps and a little in front of them. The humps are generally dark on the inner side and light on the outer. Farther backward are two or three pairs of light spots, surrounded by a darker brown area, darkest in the middle and toward the spinnerets.

The sternum is brown, and the under side of the abdomen is dark in the middle and light at the sides. In lighter individuals most of these markings can be seen, the darker ones being light brown or yellow, and the lighter ones yellow or dirty white. In some no markings can be defined.

The first pair of legs is twice as long as the second, and much longer than the fourth pair.

The terminal joint of the palpus is more than twice as long as the one before it. The palpal claw is large, with two or three teeth. Pl. xi, fig. 1*b*.

The eyes are in two rows. The upper are largest and appear still larger on account of being surrounded by dark rings. Pl. xi, figs. 1*a*, 1*b*. They are on the top of the head, the lateral pair farthest back. The front row of eyes is close to the edge of the head, just over the mandibles.

The mandibles are small and rounded forward at the base. The maxillæ are as wide as long, with the front ends nearly square. The labium is triangular. (See figure of same parts in *Hyptiotes*, Pl. xi, fig. 2*h*.)

The male is much smaller than the female. The cephalothorax is more pointed in front and lower behind. The abdomen is not much larger than the cephalothorax and not so plainly humped as in the female. The legs are but little shorter than those of the female and the markings and colors are the same. The first tibia does not have a bunch of hairs at the end like the female. The palpal organ is nearly spherical, all the parts being wound closely together. Pl. xi, figs. 1*d*, 1*e*. The femur of the male palpus has, at the base, a short process on the outer side. Pl. xi, fig. 1*f*.

The webs are round and nearly horizontal, the cross-threads usually less regular than in webs of *Epeira*. The webs are commonly made between loose stones, but sometimes in low bushes. The cocoons are half an inch long and quarter as wide, narrowed at both ends, and with numerous short points by which they are attached to the web around them. I have found them with the female under stones. The cocoons are light brown, and each female appears to make several of them. The cocoons are made in July.

This spider is found all over New England, but is not common anywhere. I have taken them from several places around Boston, Mass., in New Haven, Conn., and in Simsbury, Conn.

I have specimens of both sexes from the southern part of France, given me by Mr. E. Simon. It is found in Italy and Spain. The common *Uloborus* of the north of Europe (*U. walckenaerius*) is a very different species.

Hyptiotes Wlk. = *Mithras*, Koch.

Hyptiotes cavatus.

Pl. XI, figures 2 to 2*k*.

This peculiar spider is without much doubt the one described and figured by Hentz under the name of *Cyllopodia cavata*, although he saw but six eyes and four spinnerets, and otherwise described it incorrectly.

Its habits have been well described by B. G. Wilder in the Popular Science Monthly, 1875, where he calls it *Hyptiotes americanus.*

This spider resembles a shortened *Uloborus.* The adult female is about 4^{mm} long, and is colored like the end of one of the dead pine branches among which it usually lives.

The cephalothorax is as broad as long, highest in the middle just behind the eyes, and hollowed behind under the front of the abdomen.

The abdomen is oval, a little flattened in front. On the back are four pairs of low humps, the second largest, on each of which are a few stiff hairs.

The arrangement of the eyes resembles that in *Uloborus*, but the eyes are farther apart and farther back on the thorax. Pl. xi, figs. 2, 2*a*.

The legs are short and thickest in the middle, tapering distinctly from the patella to the claws. The hind metatarsus bearing the calamistrum is curved inward on the outer side. The claws have three or four teeth and under the claws are a few curved spines, some of which are toothed as in *Epeira.* Pl. xi, fig. 2*e*, *f*, *g*. The palpal claw has four or five teeth.

The mandibles are very small and slightly arched forward near the base.

The maxillæ and libium are like those of *Uloborus.* (Pl. XI, fif. 2*h.*)

The spinnerets are long. The cribellum is small and not divided in the middle.

The male is about half as large as the female. The abdomen is much smaller and the humps lower. The palpal organ is very large. The tube is long and slender and extends one and a half times around the organ, supported by the edge of a thin appendage. At the tip it lies against two small flexible processes and over them is a large dark horn. The whole apparatus is so large as to cover the patella as well as the tarsus. Pl. XI, figs. 2*c*, 2*d*.

The epigynum is simple externally, but the inner tubes correspond in length to those of the palpal organs.

The colors of both sexes are various shades of brown, covered with white or gray hairs. The markings on the cephalothorax and legs are usually indistinct. The eyes are surrounded by black rings. The humps on the abdomen are usually darker than the rest of the back. Dark markings follow the dorsal vessel and two or three pairs of its branches. Other individuals have the front, and some the whole back of the abdomen very dark brown.

The web consists of four rays crossed by a dozen or more threads. The point where the rays meet is attached to a thread which extends to the spider's roost, usually the end of a twig. Here it holds by the hind feet and draws the thread tight with the fore feet. When an insect strikes the web the spider lets go with the hind feet, the elasticity of the web draws the thread out with a snap, and slides the spider along it toward the web. When it reaches the center it feels the rays to find where the insect is, runs out on the nearest one, covers the prey with silk, and carries it out of the web.

The making of this web is fully described by Wilder. Having finished the rays, the spider begins with the cross threads farthest from the center, walking along the upper ray until it is near enough the next to step across, then it crosses and walks outward again on the second ray. The new cross thread is elastic enough to shorten to the proper length when she reaches the point to attach it. When the cross thread is finished to the fourth ray, the spider walks back to the center and out on the upper ray to the point for beginning another.

This spider is common all over New England and the Middle States. I have seen cocoons near their webs, like that described by Wilder, but have never traced it to them or any other spider.

EXPLANATION OF PLATES.

PLATE IX *Dictyna.*

Figs. 1, 1*a*, 1*b*, 1*c*, 1*d*, 1*e*. Dorsal markings of different individuals of *Dictyna muraria.*

Figs. 1*f*, 1*g*, Palpi of male *D. muraria.*

Fig. 2, *D. volucripes*, sternum and mouth parts: 2*a*, side of female; 2*b*, side of male; 2*c*, front of head and mandible of female; 2*d*, front of head and mandible of male; 2*e*, 2*f*, palpal organ and male tibia of different individuals.

Fig. 3, 3*a*, palpus of male *D. bostoniensis*; 3*b*, 3*c*, 3*d*, dorsal markings of *D. bostoniensis.*

Fig. 4, Tibia of male palpus of *D. longispina.*

Fig. 5, 5*a*, Male palpus of *D. minuta.*

Fig. 6, Common dorsal marking of *D. cruciata*; 6*a*, male palpus of *D. cruciata.*

Fig. 7, Palpus of male *D. rubra.*

Fig. 8, Dorsal markings of *D. volupis*; 8*a*, 8*b*, male palpus of *D. volupis*; 8*c*, tibia of male palpus seen from below.

Fig. 9, Dorsal markings of abdomen of *D. frondea*; 9*a*, palpus of male of *D. frondea.*

PLATE X. *Amaurobius.*

Fig. 1, *Amaurobius sylvestris*, × 4; 1*a*, tibia and patella of right palpus of male; 1*b*, tarsus and palpal organ; 1*c*, epigynum; 1*d*, foot; 1*e*, cribellum; 1*f*, calamistrum; 1*g*, part of web showing the arrangement of the curled threads.

Fig. 2, *Amaurobius tibialis*, epigynum: 2*a*, upper side of right palpus of male; 2*b*, outer side of the same.

Fig. 3, *Amaurobius ferox*, epigynum: 3*a*, palpal organ of male; 3*b*, tibia of male palpus, upper side; 3*c*, the same, outer side.

Fig. 4, Head of *Titanœca americana*; 4*a*, 4*b*, palpus of male; 4*c*, palpus of female; 4*d*, cribellum.

Fig. 5, Dorsal markings of *Titanœca brunnea*; 5*a*, under side of abdomen of the same; 5*b*, sternum, maxillæ and labium; 5*c*, palpus of female.

PLATE XI.

Fig. 1, *Uloborus plumipes*, side of female; 1*a*, top of cephalothorax and eyes; 1*b*, side of cephalothorax and mouth parts; 1*c*, male; 1*d*, 1*e*, palpus of male.

Fig. 2, *Hyptiotes cavatus*, × 8, female; 2*a*, the same, male; 2*b*, calamistrum; 2*c*, palpus of male; 2*d*, palpal organ from below; the place of the large terminal process is shown by a dotted line; 2*e*, *f*, *g*, first and second feet; 2*h*, labium and maxillæ; 2*i*, 2*j*, thread of web of *Hyptiotus cavatus*, showing opposite sides; 2*k*, Diagram of web.

Fig. 3, Web of *Dictyna volucripes.*

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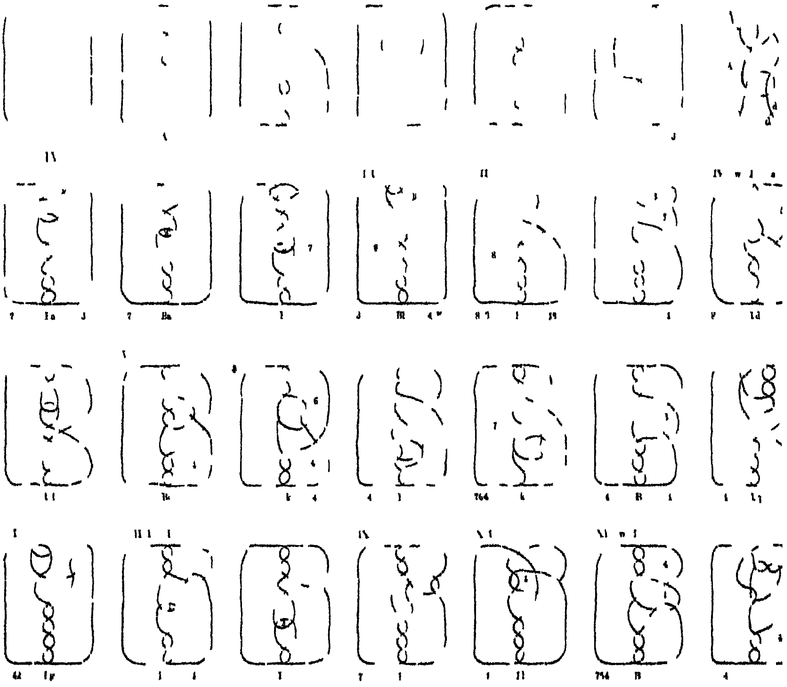
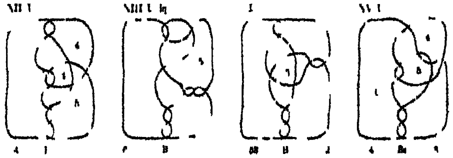




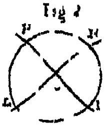
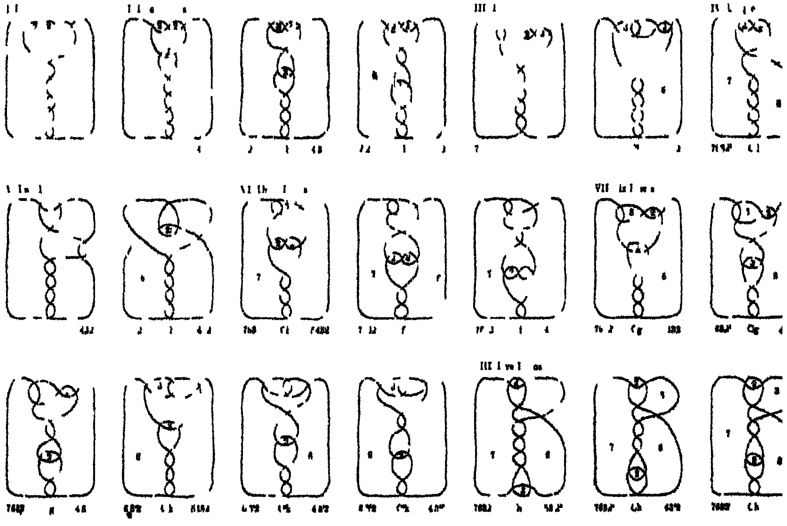
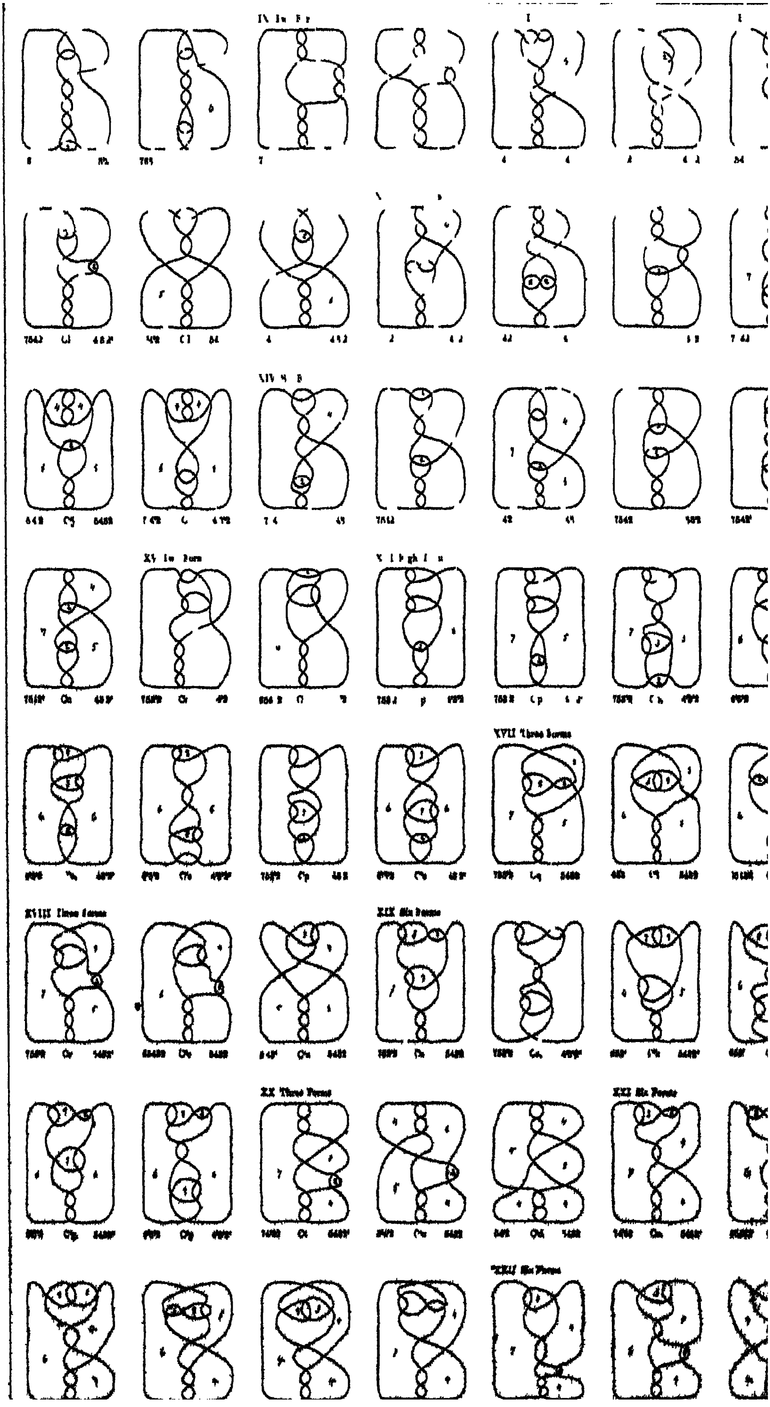


Fig 2

Class V

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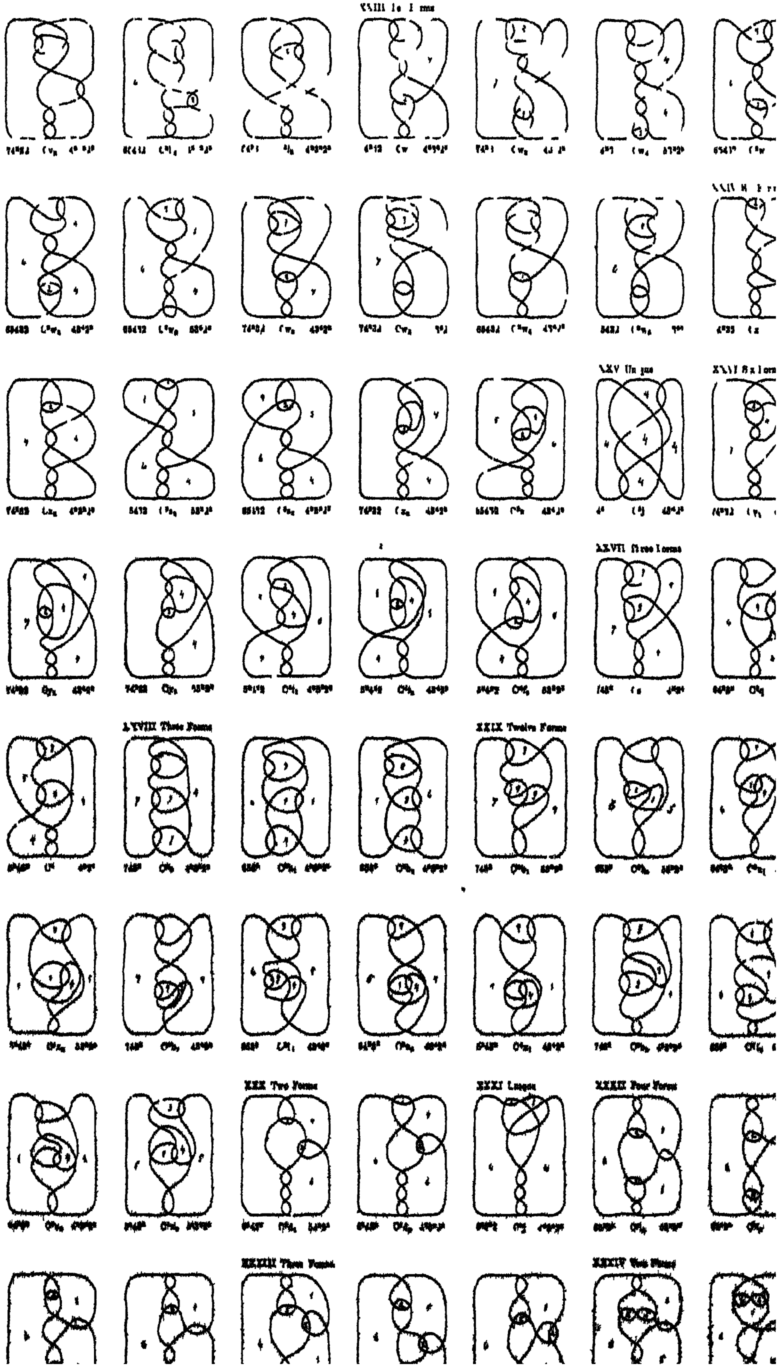
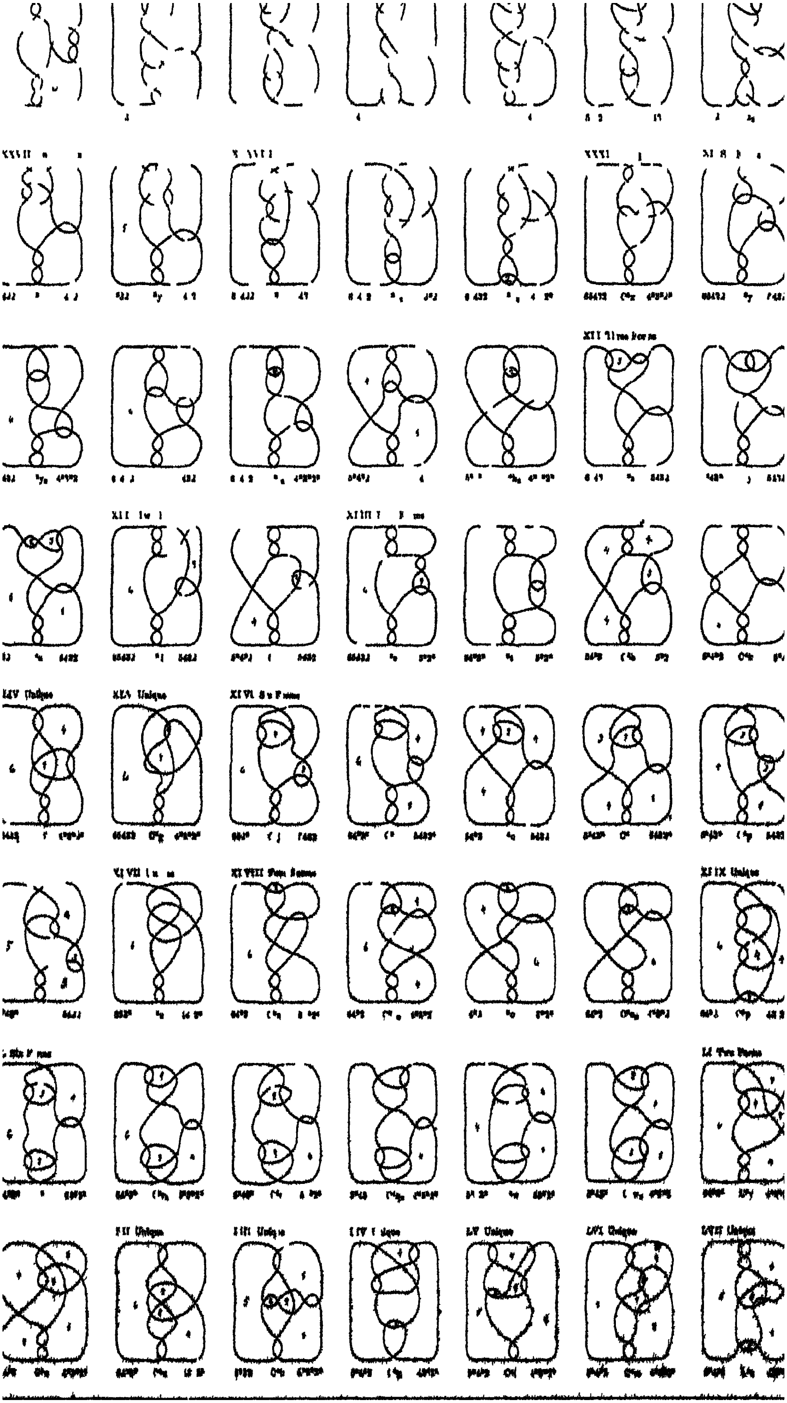
XVIII Three forms
XIX Six forms
XX Three Forms
XXI Six Forms
XXII Six Forms


XXVII Three Forms
XXVIII Three Forms
XXIX Twelve Forms
XXX Two Forms
XXXI Unique
XXXII Four Forms
XXXIII Three Forms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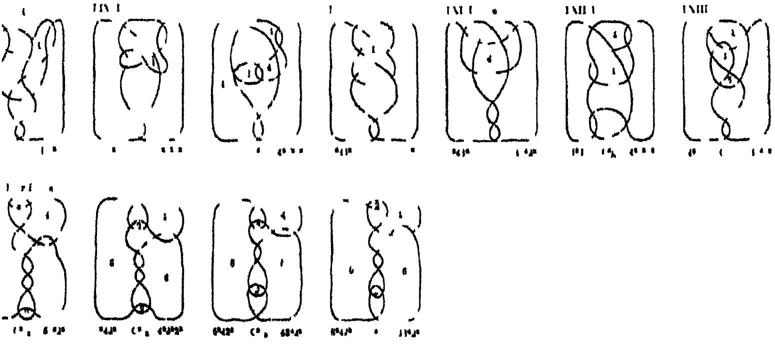
LV Unique
LVI Unique
LVII Unique



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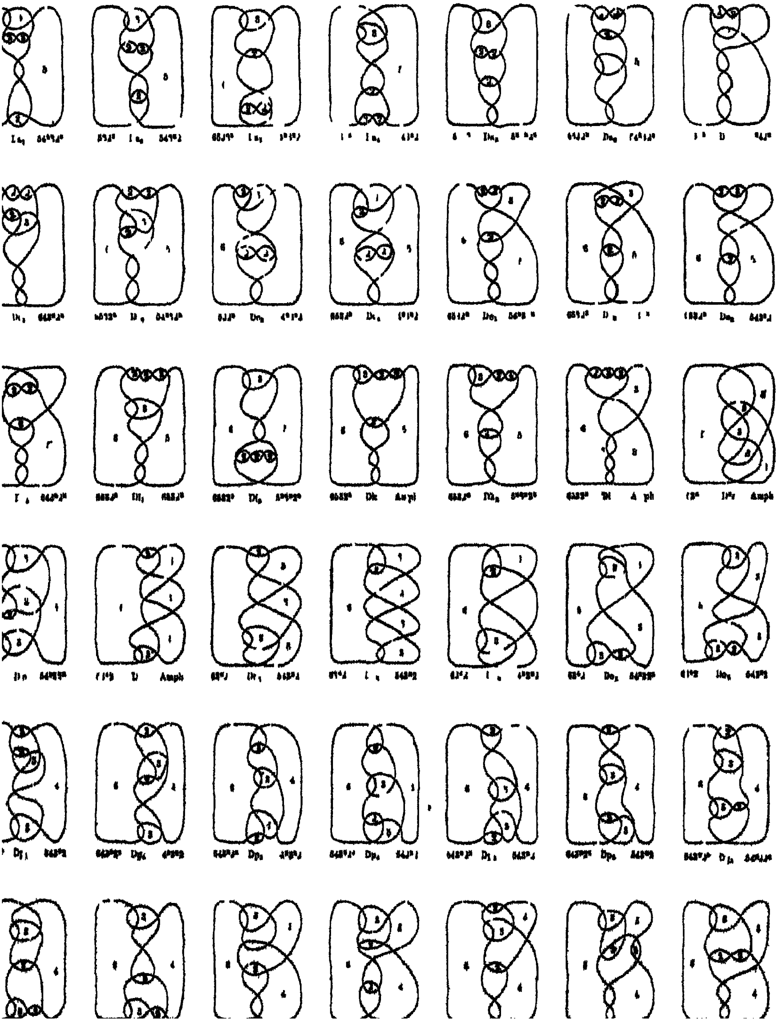

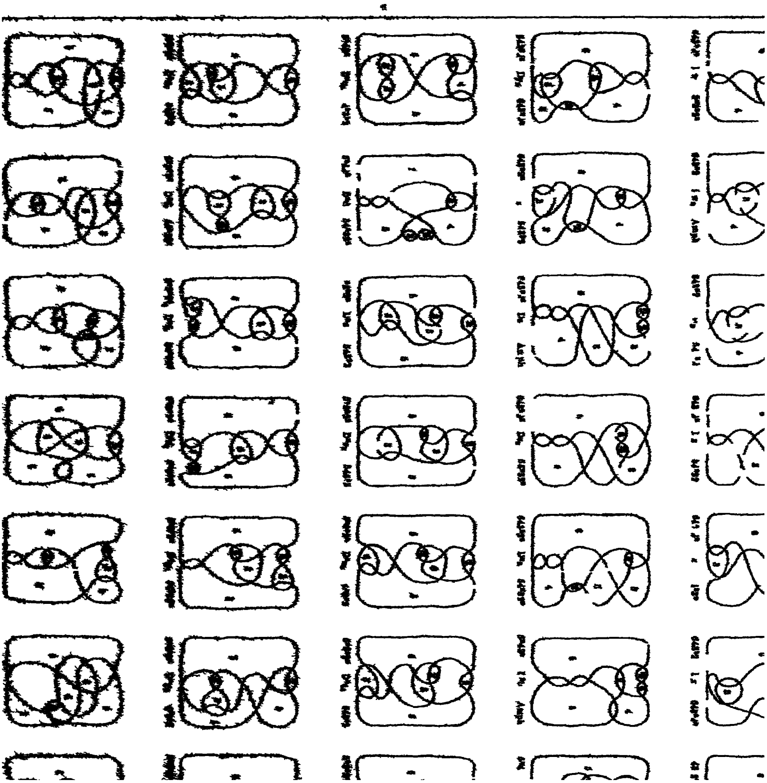

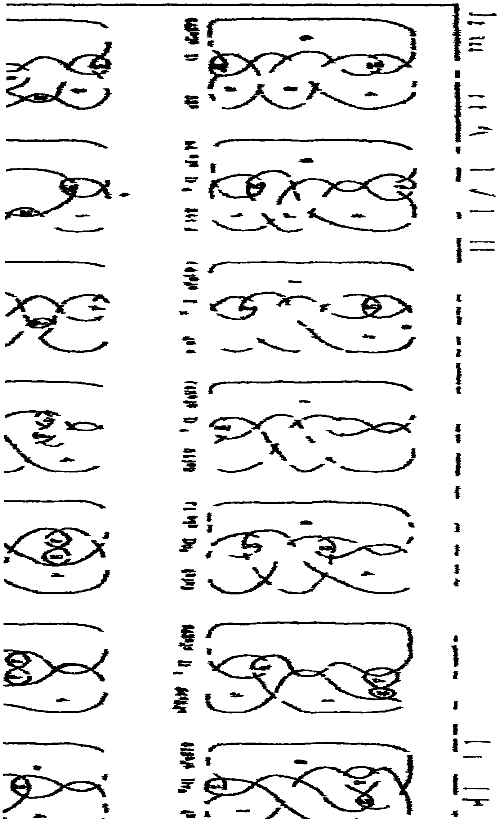

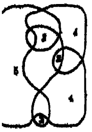
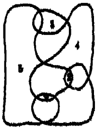
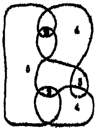
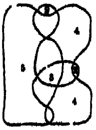
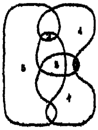
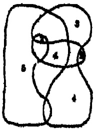
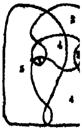
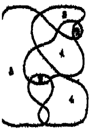
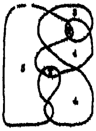
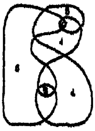
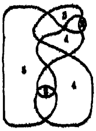
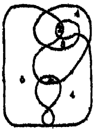
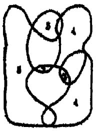
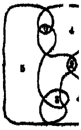
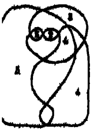
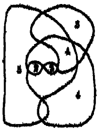
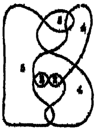
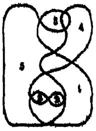
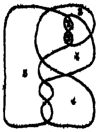
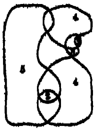
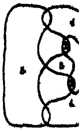
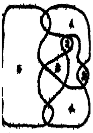
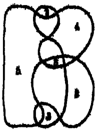
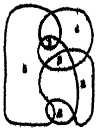
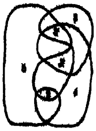
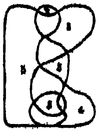
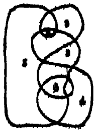
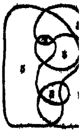
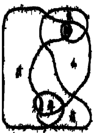
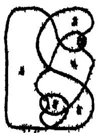
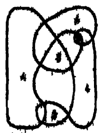
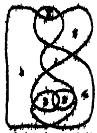
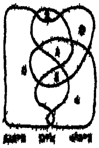
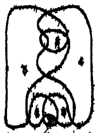
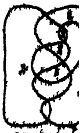

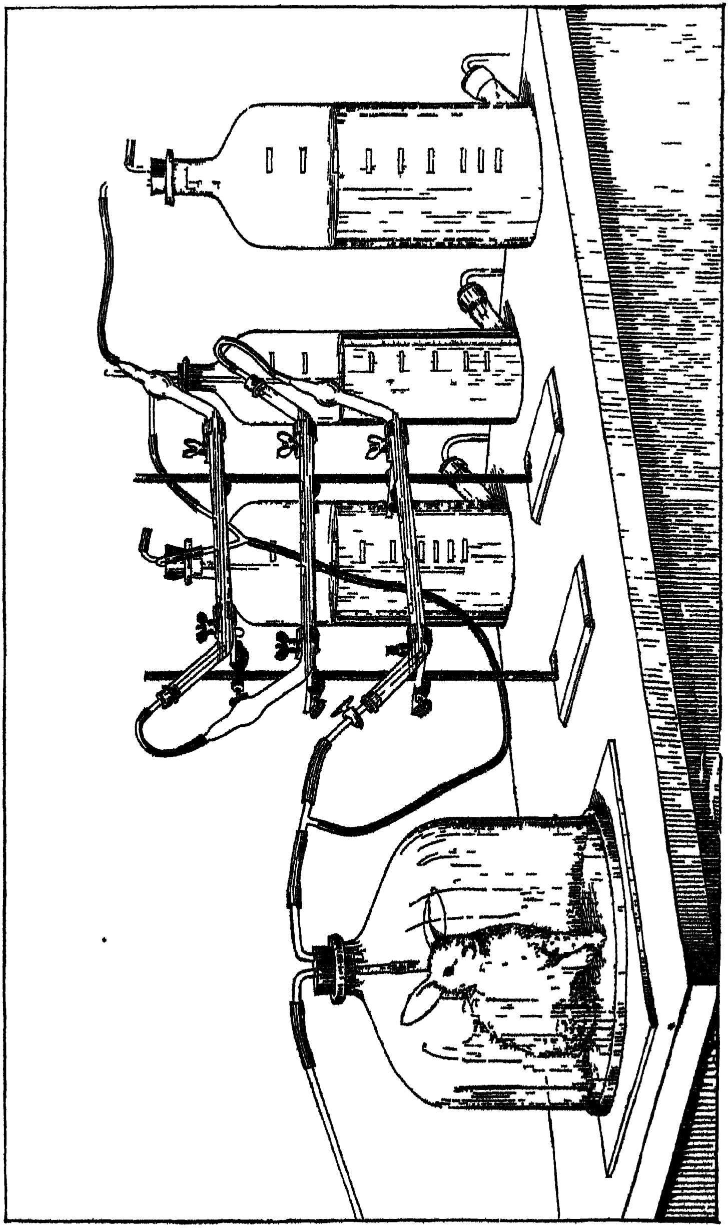

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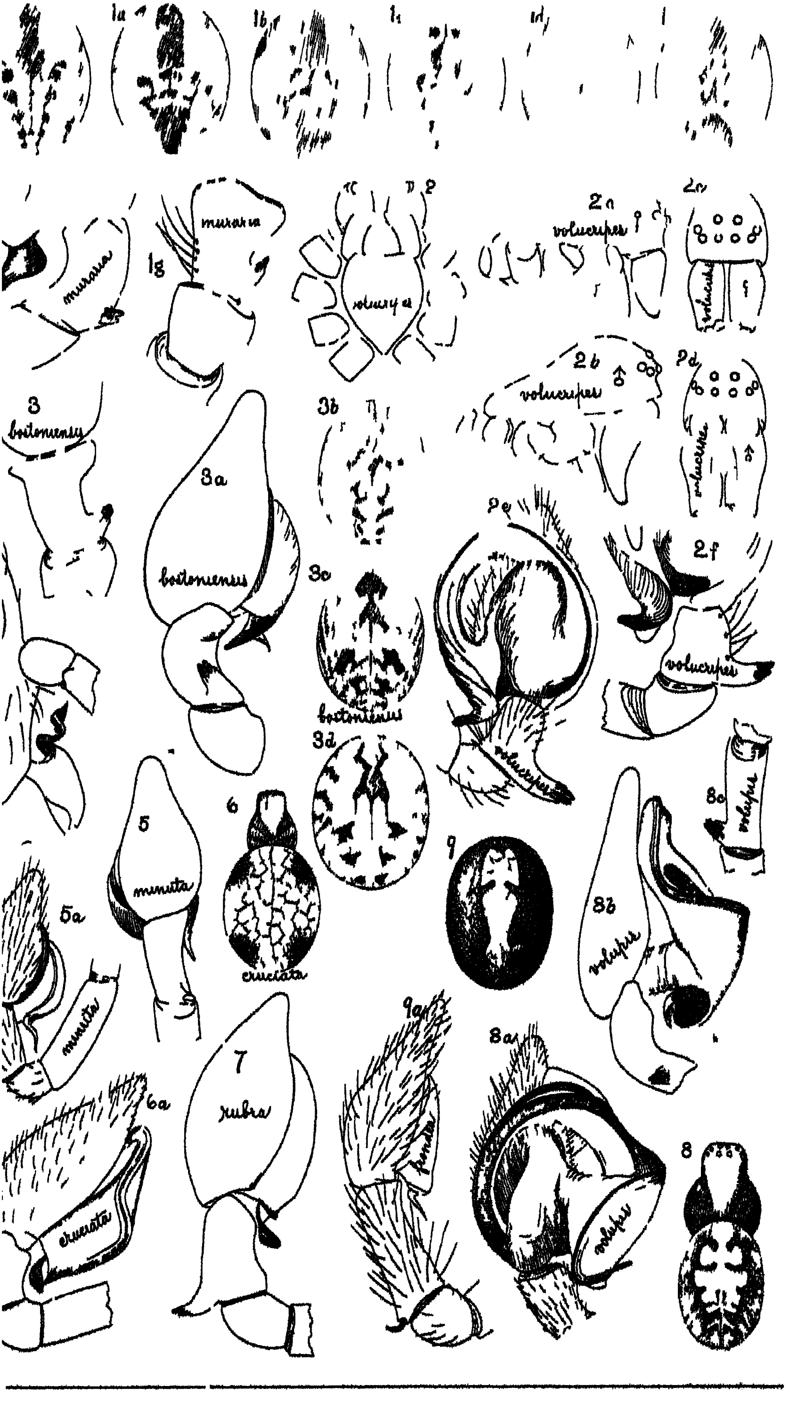
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1c
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2
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2b
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2d
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3
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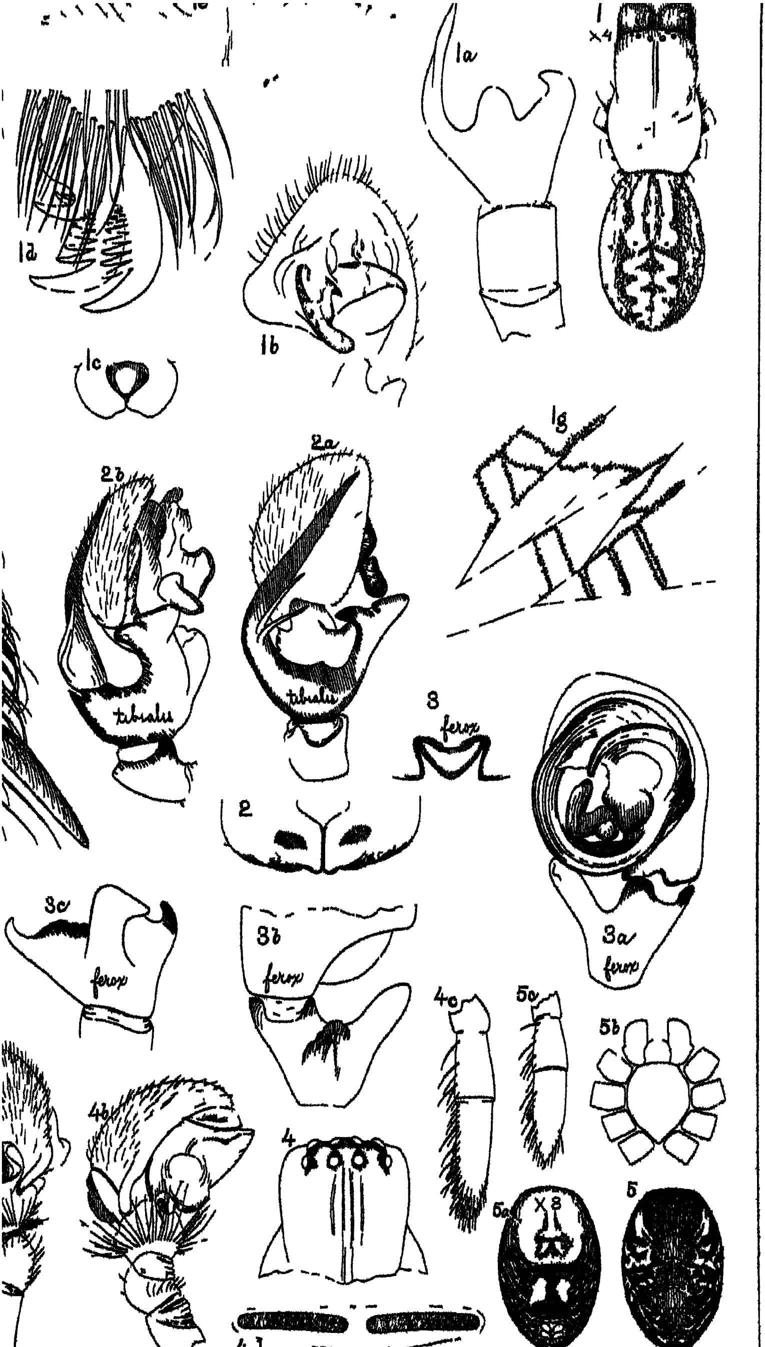

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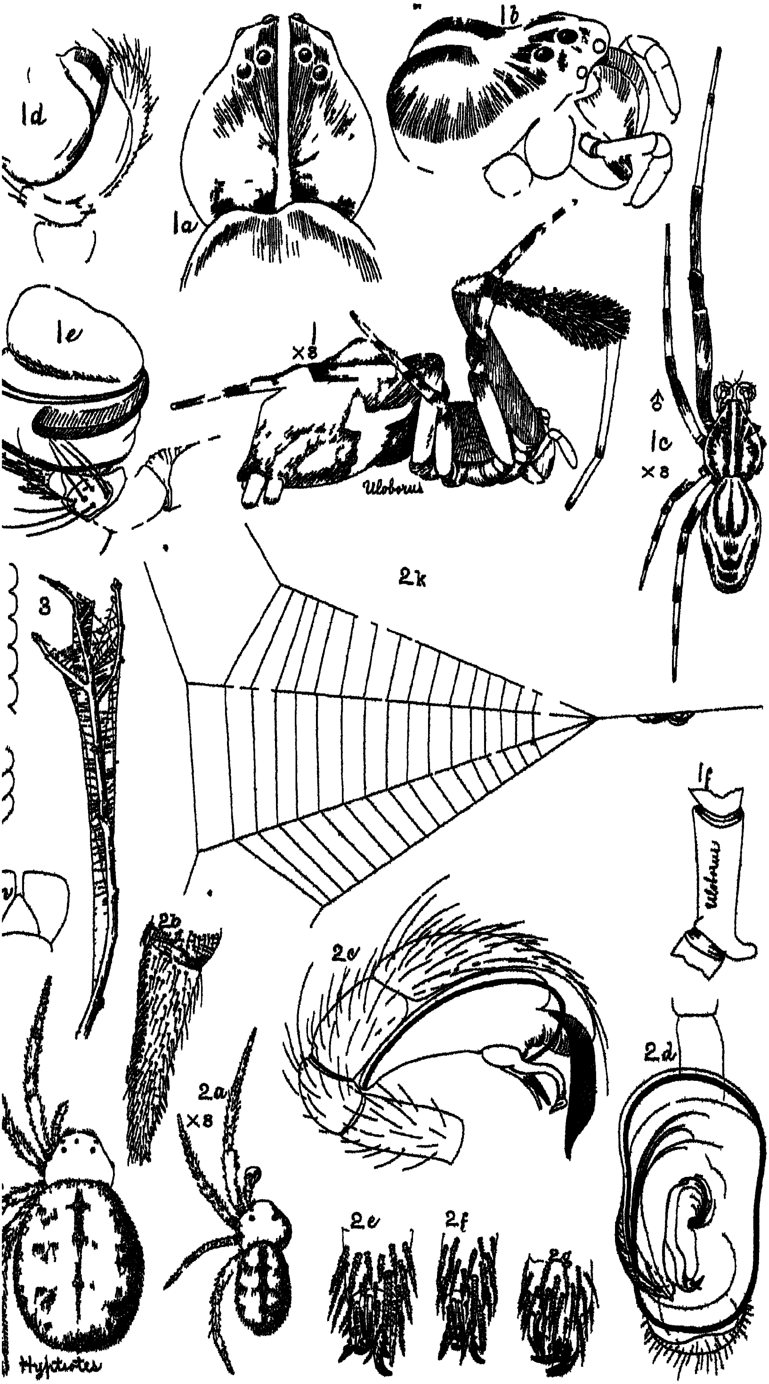

1d
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1b
1e
×8
Uloborus
♂
1c
×8
2k
3
2b
2c
1f
Uloborus
2d
2a
×8
2e
2f
2g
Hyptiotes